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Testing for Concordant Equilibria Between Population Samples

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ABSTRACT

A substantial body of theory has been developed to assess the effect of evolutionary forces on the distribution of genotypes, both single and multilocus, within populations. One area where the potential for application of this theory has not been fully appreciated concerns the extent to which population samples differ. Within populations, the divergence of genotype or haplotype frequencies from that expected under Hardy-Weinberg (HW) or linkage equilibrium can be measured as disequilibria coefficients. To assess population samples for concordant equilibria, an analytical framework for comparing disequilibria coefficients between populations is necessary. Here we present log-linear models to evaluate such hypotheses. These models have broad utility ranging from conventional population genetics to genetic epidemiology. We demonstrate the use of these log-linear models (1) as a test for genetic association with disease and (2) as a test for different levels of linkage disequilibria between human populations.

THE extent to which the varied influence of evolutionary forces such as natural selection and random genetic drift contribute to differences between population samples is of substantial interest. For example, the importance of linkage disequilibrium (LD) for population-based gene mapping approaches has focused attention on assessing the genomic distribution of LD (Huttley *et al.* 1999) and on the extent to which this distribution differs between populations (Kruglyak 1999; Lonjou *et al.* 1999). Theory predicts that the limit of LD will be greater in human populations with historically restricted sizes, giving such populations an advantage for gene mapping. Another example is the extent to which an individual's genotype at a specific locus accounts for their susceptibility to disease. Comparing population samples that differ with respect to their disease status can assess a causative disease role for variation at a locus. Although these two examples appear quite distinct, as we argue below, the effect of genetic predisposition to disease on genetic variation is analogous to the effect of natural selection in wild populations. Thus, the assessment of such seemingly disparate lines of inquiry can be unified into a single analytic framework.

To illustrate the traditional approach employed to detect genetic differentiation between groups we consider an epidemiological example where a sample is divided into groups, say "affected" and "unaffected," and tested for homogeneity of allele or genotype frequencies (see, for example, Chiano and Clayton 1998; Cox and Bell 1989; Schaid and Jacobsen 1999). Tests for genotype association implicitly allow for more complex genetic etiologies (*e.g.*, heterozygote resistance or susceptibility) than allele-based tests, but have larger degrees of freedom, and thus may have reduced statistical power. Chiano and Clayton (1998) recently proposed an additional test for association that accommodates complex genetic causation with reduced degrees of freedom relative to the genotype test. This test is restricted in its application since it makes the assumption that heterozygote genotypes do not cause disease. However, empirical evidence that this assumption is violated exists in a number of experimental systems. In one example, the F_1 progeny of a cross between inbred mouse strains NZ black and NZ white exhibit the immunological disorder lupus, which is a phenotype absent from both parental strains (Theofilopoulos and Dixon 1985).

In these traditional approaches, Hardy-Weinberg (HW) disequilibrium is treated as a confounding factor, and corrections are applied to eliminate the impact of HW departures. In addition to allele and/or genotype differences, however, it is important to understand the basis for differential departures from HW equilibrium (HWE).

The pattern of departure from HWE should reflect the underlying genetic etiology of a phenotype, suggesting that testing for HWE may also be used to assess whether a gene influences predisposition to a trait. In an epidemiological context, for example, the resistance to human immunodeficiency virus (HIV) infection of individuals homozygous for the *Δ32-CCR5* deletion allele results in a significant departure from HWE among HIV-uninfected high risk individuals, and thus an excess of *Δ32-CCR5* homozygotes in this group (Dean *et al.*

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1996). Departure from HWE in affecteds has also been used for fine scale gene mapping (Feder *et al.* 1996; Nielsen *et al.* 1999). Of course, phenomena other than selection may cause departure from HWE: admixture of genetically differentiated populations leads to a characteristic excess of homozygotes, referred to as the Wahlund effect (Hartl and Clark 1990); nonrandom mating can cause either excess homozygosity or heterozygosity; and laboratory errors stemming from difficulty in discriminating between alleles, or difficulty in sampling all alleles, can cause either excess homozygosity or heterozygosity. Departures from HWE can be measured using disequilibria coefficients (Weir 1996, p. 132).

Log-linear models present a natural framework for analysis of disequilibria coefficients between populations (Aston and Wilson 1986) and can be implemented using standard statistical software packages. We present a log-linear model approach for the comparison of single-locus disequilibria coefficients between populations. We also present log-linear modeling approaches for the comparison of disequilibria coefficients arising from nonrandom associations between loci. The latter disequilibrium is often referred to, nonrigorously, as linkage disequilibrium. While we adhere to this convention, it should be pointed out that there can be interlocus genotypic disequilibria, and that linkage is not essential for such disequilibria to occur.

STATISTICAL MODELS

A model for the effect of a selective process on genetic variation: The effect of genetic predisposition to a trait on deviations from HWE has been explored largely in the context of natural selection affecting wild populations. Here we apply analogous methodology to populations that have been divided as above into the groups "affected" and "unaffected" by selection, which we define generally as any process that differentiates individuals into two phenotypic groups on the basis of their genotypes at a locus. In Table 1 we present a simple model describing the consequences of selection on genetic variation. For a biallelic locus, having alleles A and a with frequencies $p_A + p_a = 1$, genotype frequencies prior to selection are simply those expected under HWE. In modeling a process of natural selection, differential survival of genotypes can be represented by the ratios of the fitness coefficients ω_{ij}, where i and j represent the alleles (A or a in our example). The ratios of fitness coefficients, in turn, are delimited by the corresponding selection coefficients (s_{ij}). The product of a fitness coefficient with the expected frequency (under HWE) of its corresponding genotype gives the frequency of that genotype in the unaffected group. For the sum of genotype frequencies to equal 1 in the post-selection unaffected group, frequencies are normalized by dividing each genotype's frequency in the unaffected group by the term $\bar{\omega}$. In contrast, the genotype

TABLE 1

Changes in allele and genotype frequency after selection

Group	Selection	Allele		Genotype		
		p_A	p_a	P_{AA}	P_{Aa}	P_{aa}
	Frequency (pre)	p_A	p_a	p_A^2	$2p_Ap_a$	p_a^2
	Fitness			$\omega_{AA} = 1 - s_{AA}$	$\omega_{Aa} = 1 - s_{Aa}$	$\omega_{aa} = 1 - s_{aa}$
Unaffected	Frequency (post)	$p_A' = (p_A^2\omega_{AA} + p_Ap_a\,\omega_{Aa})/\bar{\omega}$[a]	$p_a' = (p_a^2\omega_{aa} + p_Ap_a\,\omega_{Aa})/\bar{\omega}$	$p_A^2\omega_{AA}/\bar{\omega}$	$2p_Ap_a\omega_{Aa}/\bar{\omega}$	$p_a^2\omega_{aa}/\bar{\omega}$
Affected		$p_A'' = (p_A^2 s_{AA} + p_Ap_a s_{Aa})/\bar{s}$[a]	$p_a'' = (p_a^2 s_{aa} + p_Ap_a s_{Aa})/\bar{s}$	$p_A^2 s_{AA}/\bar{s}$	$2p_Ap_a s_{Aa}/\bar{s}$	$p_a^2 s_{aa}/\bar{s}$

See text for detailed explanation.

[a] $\bar{\omega}$ is the mean fitness of the unaffected group and equals $p_A^2\omega_{AA} + 2p_Ap_a\omega_{Aa} + p_a^2\omega_{aa}$. $\bar{s}$ is the equivalent for the affected group and equals $p_A^2 s_{AA} + 2p_Ap_a s_{Aa} + p_a^2 s_{aa} = 1 - \bar{\omega}$.

frequencies in the affected group are governed by the ratio of selection coefficients. In the standard context of natural selection in wild populations, the affected group is taken to be the nonsurvivors and hence ignored, with differences between unaffected groups from population to population being of primary interest. In the context of genetic epidemiology, interest centers on comparisons of affected with unaffected groups, and selection coefficients may be equated with penetrances. So s_{ij} is the probability of a phenotype given genotype ij and $\omega_{ij} + s_{ij} = 1$. Hence, ω_{ij} will then be the probability of not having a phenotype given genotype ij. Beyond affected and unaffected groups, any two or more groups undergoing different selection regimes can also be distinguished by their ω and s coefficients.

Because genotype frequencies are determined by the ω_{ij} in the unaffected group and s_{ij} in the affected group, variation at a causative locus will exhibit differential departures from HWE in the affected and unaffected groups (Table 1). This suggests a novel null hypothesis for comparing affected with unaffected groups: that the disequilibrium coefficient(s) at a locus are the same in affecteds and unaffecteds. Both the traditional allele and genotype association tests are indirect assessments of this null hypothesis.

Below we present log-linear models for tests of concordant equilibria. Although the models and examples considered are for biallelic loci, multiallelic loci can be readily accommodated. Examples of applying the models presented here are available at http://cbis.anu.edu.au/publications.html as generalized linear interactive modeling (GLIM), SAS, or R transcript files.

Testing groups for concordant equilibria at a single locus: Testing for concordance with HWE in a sample is predominantly performed using an additive statistical model. Consider a biallelic locus with alleles A and a, genotypic frequencies P_{AA}, P_{Aa}, P_{aa}, and allele frequencies $p_A + p_a = 1$. Departure from equilibrium expectation is commonly evaluated by whether a disequilibrium coefficient, namely $D = P_{AA} - p_A^2$, differs from 0. Alternatively, the testing of HWE using log-linear modeling assumes a multiplicative model (additive on the log scale) with departures from HW being evaluated by whether the coefficient $M_{AA} = 4P_{AA}P_{aa}/P_{Aa}^2$ differs from 1 (Weir 1996, p. 104). A log-linear framework greatly facilitates the analysis of more complex models as existing software packages can be used. To test n groups for concordant equilibria, additional terms are included in the basic log-linear model. There are several parameterizations for the log of expected genotype frequencies in group i under the full, or saturated, log-linear model. The one we use here can be written as

$$\ln(P_{AA})_i = \ln M + \ln \tau_i + 2\ln M_A + \ln M_{AA} + 2\ln(\tau M_A)_i + \ln(\tau M_{AA})_i$$

$$\ln(P_{Aa})_i = \ln 2 + \ln M + \ln \tau_i + \ln M_A + \ln(\tau M_A)_i$$

$$\ln(P_{aa})_i = \ln M + \ln \tau_i,$$

where $i = 1, 2, \ldots n$ for the n groups. The parameter M is a mean effect and so common to all genotypes; τ represents the possible unequal sizes of samples from group to group; M_A represents the frequency of allele A compared with allele a in the total sample; M_{AA} represents the HW (monogenic) disequilibrium coefficient in the total sample; (τM_A) represents the unequal allele frequencies between groups; and (τM_{AA}), the differential monogenic disequilibria between groups. Removing all terms containing τ reduces these equations to those presented in Weir (1996).

Disequilibria coefficients from the multiplicative and additive statistical frameworks have different properties: The disequilibrium coefficient of the additive model, D, is a function of the three fitness coefficients and the allele frequency, *e.g.*, from the unaffected group $D = p_A^2 p_a^2(\omega_{AA}\omega_{aa} - \omega_{Aa}^2)/\overline{\omega}^2$. In some instances the D coefficients from the unaffected and affected groups may be equal. One circumstance when this can occur is if $\omega_{AA} = 1$ and $\omega_{aa} = 0$. Under these constraints, D in both the affected and unaffected samples will be equal when $\omega_{Aa} = p_A^2/(2p_A^2 - 2p_A + 1)$. More generally, D in the two groups will be equal when $\omega_{AA} = 1$ and the following complex function is true:

$$p_A = \frac{1}{(\omega_{aa} - 2\omega_{Aa} + 1)^2} \times \left((\omega_{aa} - \omega_{Aa})(\omega_{aa} - 2\omega_{Aa} + 1) + \sqrt{((\omega_{aa} - 2\omega_{Aa} + 1)^2\sqrt{((\omega_{Aa}^2 - \omega_{aa})(\omega_{Aa}^2 - 2\omega_{Aa} + 1))})}\right).$$

In contrast, the multiplicative coefficient M_{AA} is independent of allele frequency [$M_{AA} = \omega_{AA}\omega_{aa}/\omega_{Aa}^2$ in the unaffected group and $M_{AA} = (1 - \omega_{AA})(1 - \omega_{aa})/(1 - \omega_{Aa})^2$ in the affected group]. Given our formulation of $0 \leq \omega_{ij} \leq 1$, the multiplicative coefficients in the two groups will be equal only when $\omega_{Aa} = \omega_{Aa} = \omega_{aa} = 1/2$, where the genotypes do not differ in their probability of exhibiting a phenotype.

In addition to measuring the difference in disequilibria between two samples, the above model also assesses the extent to which two samples are differentiated by allele frequency. In the fully saturated model, allele frequency is evaluated as $M_A = P_{Aa}/(2P_{aa})$ (Weir 1996). This can be reformulated as $(p_A\omega_{Aa})/(p_a\omega_{aa})$ for the unaffected sample and $[p_A(1 - \omega_{Aa})]/[p_a(1 - \omega_{aa})]$ for the affected sample. From these equations it can be seen that M_A of the two samples will be equal when $\omega_{Aa} = \omega_{aa}$. It is important to note that the τM_A term could still be significant when this relationship is true.

Example test of groups for concordant equilibria at a single locus: The log-linear models were implemented in GLIM. We illustrate application of the single-locus model to $+/\Delta 32$ *CCR5* genotype data from longitudinal

TABLE 2

Observed and expected +/Δ*32* *CCR5* genotype frequencies from Europeans

Population	Genotype	Observed[a]	Expected	Residuals[b]
	+/+	691	701.2	−0.38
HIV+	+/Δ*32*	186	165.7	1.58
	Δ*32*/Δ*32*	0[c]	10.7	−3.11
	+/+	468	457.8	0.48
HIV−	+/Δ*32*	87	107.3	−1.96
	Δ*32*/Δ*32*	17	6.8	3.88

[a] Data are the sum of genotype frequencies for all cohorts except the ALIVE cohort from Table 2 of Dean *et al.* (1996).

[b] Pearson's residual (Francis *et al.* 1993), calculated as (Observed − Expected)/$\sqrt{\text{Expected}}$. Residuals are estimated from a model without the τM_{AA} term, model 5, Table 3.

[c] Taken to be 0.5.

AIDS cohorts (Dean *et al.* 1996). We include only homosexual men from the DCG, MAC, and SFCC cohorts. The frequencies of genotypes in each group are presented in Table 2. HIV− refers to individuals who have not contracted, but are at risk for exposure to, HIV (the resistant group). HIV+ refers to individuals that have contracted HIV (the susceptible group). There are four independent parameters in the model and the complete sequential addition of model terms is shown in Table 3. Parameters are estimated by maximum likelihood assuming a multinomial sampling of genotypes. The fit of a model is measured as the likelihood-ratio test statistic or deviance $\mathfrak{D} = -2\ln(L_c/L_f)$, where L_c is the likelihood estimated under the model of interest and L_f the likelihood estimated under the full model (Francis *et al.* 1993). The significance for inclusion of an individual term in the model is assessed by comparing the deviances of the model with and without the term. The difference in deviances, $\Delta\mathfrak{D}$, of the two models is distributed approximately as χ^2 with degrees of freedom equal to the difference in degrees of freedom of the two models (Δd.f.).

When the full hierarchy of models is being considered there are some general principles that can be employed to guide interpretation. First, a nonsignificant residual deviance for model 4 does not mean that both interaction terms (τM_A, τM_{AA}) are not significant. This principle also applies to the two-locus models. Second, for Pearson residuals, calculated as (Observed − Expected)/$\sqrt{\text{Expected}}$, an absolute value greater than two indicates lack of fit for those categories.

The significance of group (τ) and allele (M_A) terms indicates that the HIV− and HIV+ groups are unequal in size, and in the combined sample the + and Δ*32* alleles are unequal in frequency. The term M_{AA} does not contribute significantly to this model, consistent with the combined affecteds and unaffecteds being drawn from a population in HWE. The group-by-allele interaction term (τM_A) is also not significant, indicating no difference in allele frequency between groups. We note that because the model adjusts for departures from HW, the group-by-allele interaction term under this model will be different from that resulting from the "standard" allele frequency goodness-of-fit test. The group-by-monogenic disequilibria interaction term in the model is highly significant. This result indicates that group-specific HW disequilibria (*i.e.*, τM_{AA}) coefficients significantly improve the fit of the log-linear model. A two-tailed Fisher's exact test on the genotype distribution was also highly significant ($P \leq 10^{-8}$), affirming the validity of the asymptotic approximation used for $\Delta\mathfrak{D}$. While the samples presented in Table 2 contain a

TABLE 3

Log-linear analysis of the *CCR5* locus in HIV−/+ populations

Model	Effects in model[a]	d.f.	Deviance	$\Delta\mathfrak{D}$	Δd.f.
1	M	5	2155.5*	—	—
2	M, τ	4	2090.6	64.9*	1
3	M, τ, M_A	3	34.9	2056.0*	1
4	M, τ, M_A, M_{AA}	2	34.8	0.10	1
5	M, τ, M_A, M_{AA}, (τM_A)	1	34.8	0.004	1
6	M, τ, M_A, M_{AA}, (τM_A), (τM_{AA})	0	0.0	34.75**	1

* Significantly different from 0 ($P < 0.01$).

[a] Effects in model are described in the text.

small proportion of individuals of non-European ancestry, restricting the analysis to Europeans still results in rejecting the null hypothesis (results not shown). An examination of the residuals from the model without the τM_{AA} term (Table 2) reveals an excess of $\Delta 32/\Delta 32$ homozygotes in the HIV− group and deficit in the HIV+ group. This is consistent with the hypothesized resistance to HIV infection conferred by absence of the CCR5 receptor (Dean *et al.* 1996).

Testing groups for concordant equilibria at two loci: As for HW, departures from linkage equilibrium may arise from a number of evolutionary processes: selection, random genetic drift, nonrandom mating, admixture of genetically differentiated populations, and mutation. There is also a substantial body of theory concerning the distributional properties of the disequilibria coefficients that can exist between two loci (see Weir 1996).

Data for assessing the occurrence of nonrandomness between loci can take two general forms: (1) phase-known data, where specific chromosomal or gametic combinations of variants are explicitly known; and (2) genotypic data with phase unknown. We treat each class of data separately.

Consider phase-known data of two loci with alleles A/a and B/b from n groups. Under the full model the log of expected frequencies of the four possible gametes in group i ($i = 1, 2, \ldots n$) can be parameterized as

$$\ln(P_{AB})_i = \ln M + \ln \tau_i + \ln M_A + \ln M_B + \ln M_{AB} + \ln(\tau M_A)_i + \ln(\tau M_B)_i + \ln(\tau M_{AB})_i$$

$$\ln(P_{Ab})_i = \ln M + \ln \tau_i + \ln M_A + \ln(\tau M_A)_i$$

$$\ln(P_{aB})_i = \ln M + \ln \tau_i + \ln M_B + \ln(\tau M_B)_i$$

$$\ln(P_{ab})_i = \ln M + \ln \tau_i,$$

where M is a mean effect and so common to all gamete combinations; τ and M_A are as above; M_B represents the frequency of allele B compared with allele b in the total sample, and M_{AB} represents the digenic disequilibria (intragametic nonindependence) of alleles at the two loci in the sample; (τM_A) and (τM_B) represent the differential allele frequency between groups at loci A and B, respectively; and (τM_{AB}) the between-group difference in digenic disequilibria.

Analyzing phase-unknown genotypic data for disequilibria is more complex and involves numerous disequilibria terms. Assuming phase for double heterozygotes is unknown and that only nine genotypic classes can be distinguished, under the full model the log of genotype frequencies can be expressed as

$$\ln(P^{AB}_{AB})_i = \ln M + \ln\tau_i + \ln M^2_A + \ln M^2_B + \ln M_{AA} + \ln M_{BB} + \ln Q^2_{AB} + \ln M^2_{AAB} + \ln M^2_{ABB} + \ln M^{AB}_{AB} + \ln(\tau M^2_A)_i + \ln(\tau M^2_B)_i + \ln(\tau M_{AA})_i + \ln(\tau M_{BB})_i + \ln(\tau Q^2_{AB})_i + \ln(\tau M^2_{AAB})_i + \ln(\tau M^2_{ABB})_i + \ln(\tau M^{AB}_{AB})_i$$

$$\ln(P^{AB}_{Ab})_i = \ln 2 + \ln M + \ln \tau_i + \ln M^2_A + \ln M_B + \ln M_{AA} + \ln Q^2_{AB} + \ln M_{AAB} + \ln(\tau M^2_A)_i + \ln(\tau M_B)_i + \ln(\tau M_{AA})_i + \ln(\tau Q^2_{AB})_i + \ln(\tau M_{AAB})_i$$

$$\ln(P^{Ab}_{Ab})_i = \ln M + \ln \tau_i + \ln M^2_A + \ln M_{AA} + \ln(\tau M^2_A)_i + \ln(\tau M_{AA})_i$$

$$\ln(P^{AB}_{aB})_i = \ln 2 + \ln M + \ln \tau_i + \ln M_A + \ln M^2_B + \ln M_{BB} + \ln Q^2_{AB} + \ln M_{ABB} + \ln(\tau M_A)_i + \ln(\tau M^2_B)_i + \ln(\tau M_{BB})_i + \ln(\tau Q^2_{AB})_i + \ln(\tau M_{ABB})_i$$

$$\ln(P^{AB}_{ab} + P^{Ab}_{aB})_i = \ln 4 + \ln M + \ln \tau_i + \ln M_A + \ln M_B + \ln S_{AB} + \ln(\tau M_A)_i + \ln(\tau M_B)_i + \ln(\tau S_{AB})_i$$

$$\ln(P^{Ab}_{ab})_i = \ln 2 + \ln M + \ln \tau_i + \ln M_A + \ln(\tau M_A)_i$$

$$\ln(P^{aB}_{aB})_i = \ln M + \ln \tau_i + \ln M^2_B + \ln M_{BB} + \ln(\tau M^2_B)_i + \ln(\tau M_{BB})_i$$

$$\ln(P^{ab}_{aB})_i = \ln 2 + \ln M + \ln \tau_i + \ln M_B + \ln(\tau M_B)_i$$

$$\ln(P^{ab}_{ab})_i = \ln M + \ln \tau_i.$$

In this model, M is a mean effect and so common to all bilocus genotypic combinations; τ, M_A, and M_B are the same as for the phase-known model above; M_{AA} and M_{BB} are the monogenic disequilibrium coefficients for each locus in the total sample; $S_{AB} = (M_{AB} + M_{A/B})/2$ is the sum of digenic disequilibria for the total sample; $Q_{AB} = (M_{AB}M_{A/B})$ is the product of digenic disequilibria for the total sample; M_{AAB} and M_{ABB} are the trigenic disequilibria for the total sample; and M^{AB}_{AB} the quadrigenic disequilibria for the total sample (Weir and Wilson 1986). All terms in the model involving τ represent differences between groups, as for the models described above.

If gametic phase is known, it may be desirable to explicitly evaluate all disequilibria terms. In this case, the terms S_{AB} and Q_{AB} can be replaced by M_{AB} and $M_{A/B}$ (Weir and Wilson 1986). The latter two terms represent the intra- and intergametic digenic disequilibria, respectively.

Example test of groups for concordant equilibria at two loci, phase unknown: Because the equations presented for phase-unknown data above are overparameterized we assume no quadrigenic disequilibria and set the term $M^{AB}_{AB} = 1$. An application of this model to genotypic counts data from two groups for MN and S blood group loci (Mourant *et al.* 1976) is presented (see Table 4). These genes are closely linked on chromosome 4 (Race and Sanger 1975). The sequential addi-

TABLE 4

Observed genotype frequencies at *MN* and *S* loci from two populations with residuals from three of the models

	Genotypes			Residuals[a]		
Population	*MN*	*S*	Observed	Model 14[b]	Model 15	Model 16
Xavante Indians	*MM*	*SS*	91	−1.15	−0.75	0.49
	MM	*Ss*	147	0.40	−0.62	−0.38
	MM	*ss*	85	0.78	1.82	0.02
	MN	*SS*	32	1.81	0.86	−0.72
	MN	*Ss*	78	−1.47	0.00	0.00
	MN	*ss*	75	0.64	−0.51	0.52
	NN	*SS*	5	2.04	2.07	−0.08
	NN	*Ss*	17	3.35	2.55	1.34
	NN	*ss*	7	−2.80	−2.54	−1.33
Irish Republic	*MM*	*SS*	121	1.12	0.70	−0.40
	MM	*Ss*	248	−0.30	0.50	0.30
	MM	*ss*	164	−0.53	−1.14	−0.01
	MN	*SS*	53	−1.11	−0.60	0.62
	MN	*Ss*	422	0.70	0.00	0.00
	MN	*ss*	375	−0.27	0.23	−0.22
	NN	*SS*	9	−0.85	−0.85	0.06
	NN	*Ss*	65	−1.08	−0.91	−0.56
	NN	*ss*	241	0.81	0.71	0.29

[a] Pearson's residual (Francis *et al.* 1993).
[b] Models are indicated in Table 5.

tion of terms to the model is presented in Table 5. As in the single-locus application, the significance of the τ, M_A, and M_B terms reflects unequal frequencies of groups and alleles. Significance of the monogenic disequilibria term M_{BB} and digenic disequilibria terms S_{AB} and Q_{AB} plausibly arises from both the within-group linkage disequilibrium between these loci (Weir and Wilson 1986) and pooling the genetically differentiated groups. This interpretation is supported by the highly significant τM_A and τM_B group-by-allele interaction terms. Interestingly, both group-by-digenic disequilibria interaction terms are highly significant, while the trigenic disequilibria τM_{ABB} term is nominally significant. These latter observations suggest that the relative contributions of evolutionary forces affecting the interactions of alleles at these two loci significantly differ between these populations. An examination of the residuals from models 14–16 indicated that the Xavante Indian population exhibits the greatest departure from expectation. In particular, Xavante Indian *NNss* individuals are in strong deficit. The role of intra- (M_{AB}) or intergametic ($M_{A/B}$) disequilibria in the significance of the digenic disequilibria terms cannot be distinguished as they are confounded within the terms S_{AB} and Q_{AB}. It seems plausible, however, that this difference may result partly from intragametic disequilibrium since smaller effective population sizes, presumably an attribute of the traditionally hunter-gatherer Xavante Indians (Maybury-Lewis 1971, p. 35) relative to the agrarian Irish Republic population, can lead to high levels of disequilibrium from random genetic drift (Slatkin 1994). Interestingly, the significance of the trigenic disequilibrium term (τM_{ABB}), resulting from the deficit of Xavante Indian *NNss* individuals, may indicate the differential operation of natural selection in these two populations.

DISCUSSION

Tests of genetic differentiation have commonly involved directly comparing allele and/or genotype frequencies between groups, with possible adjustment for multiple testing. We have noted that an outcome of selective processes is the differential departures from population genetic equilibria at causative genes in affected and unaffected groups. Here, approaches have been presented to directly test the null hypothesis that the disequilibria coefficients in different groups are the same. To discuss the attributes of these tests we focus primarily on the application of testing for genetic association.

Properties of the single-locus test: The single-locus test for concordant equilibria between groups has several advantages over standard association tests. The log-linear models allow partitioning of the differentiation between samples into the contributions of alleles and interallelic interaction. These contributions are confounded in the conventional genotype goodness-of-fit test. Disentangling these effects enables explicit assessment of hypotheses concerning their roles, reducing the degrees of freedom for tests of complex genetic etiologies. For biallelic loci the degrees of freedom for the concordant equilibria test are equal to that for allele

TABLE 5

Log-linear analysis of the *MN* and *S* loci from two populations

Model	Effects in model[a]	d.f.	Deviance	$\Delta\mathfrak{D}$	Δd.f.
1	M	17	1724.10**	—	—
2	M, τ	16	1090.40	633.7**	1
3a	M, τ, M_A	15	853.70	236.68**	1
3b	M, τ, M_B	15	723.40	367.00**	1
4	M, τ, M_A, M_B	14	486.72	603.48**	2
5a	M, τ, M_A, M_B, M_{AA}	13	485.59	1.13	1
5b	M, τ, M_A, M_B, M_{BB}	13	481.45	5.27*	1
6	M, τ, M_A, M_B, M_{AA}, M_{BB}	12	480.31	6.41*	2
7	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}	11	463.76	16.35**	1
8	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}	10	218.42	245.30**	1
9a	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}	9	217.77	0.66	1
9b	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{AAB}	9	218.36	0.07	1
10	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}	8	217.15	1.27	2
11a	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_A)	7	58.21	158.90**	1
11b	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_B)	7	152.33	64.32**	1
12	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_A), (τM_B)	6	37.94	179.21**	2
13a	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_A), (τM_B), (τM_{AA})	5	37.50	0.44	1
13b	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_A), (τM_B), (τM_{BB})	5	35.71	2.22	1
14	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_A), (τM_B), (τM_{AA}), (τM_{BB})	4	35.46	2.49	2
15	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_A), (τM_B), (τM_{AA}), (τM_{BB}), (τS_{AB})	3	26.93	9.16**	1
16	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_A), (τM_B), (τM_{AA}), (τM_{BB}), (τS_{AB}), (τQ_{AB})	2	5.94	20.36**	1
17a	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_A), (τM_B), (τM_{AA}), (τM_{BB}), (τS_{AB}), (τQ_{AB}), (τM_{ABB})	1	1.62	4.38*	1
17b	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_A), (τM_B), (τM_{AA}), (τM_{BB}), (τS_{AB}), (τQ_{AB}), (τM_{AAB})	1	2.89	3.05	1
18	M, τ, M_A, M_B, M_{AA}, M_{BB}, S_{AB}, Q_{AB}, M_{ABB}, M_{AAB}, (τM_A), (τM_B), (τM_{AA}), (τM_{BB}), (τS_{AB}), (τQ_{AB}), (τM_{ABB}), (τM_{AAB})	0	0.00	5.94	2

* Significantly different from 0 ($P < 0.05$); ** significantly different from 0 ($P < 0.01$).
[a] Effects in model are described in the text.

association tests and are intermediate between those for allele and genotype association tests for multiallelic loci. Moreover, the test for concordant equilibria can detect genetic associations where the underlying genetic causation is complex. Alternatively, the full hierarchy of models (Table 3, models 4–6) may be considered and the basis for rejection of the null hypothesis can be identified. The latter approach avoids the necessity of applying a multiple test correction that arises when both allele and genotype association tests are performed. In our example, after rejecting the null hypothesis for model 4 (Table 3) we see that the genotype distribution difference arises not from allele frequency differences but as a consequence of the differential frequency of $\Delta 32/\Delta 32$ homozygotes in the two groups.

The log-linear approach should provide improvements in power relative to the conventional association tests. The conventional genotype goodness-of-fit test is a special case of the log-linear model—deviance ($\mathfrak{D}$) from model 4 and the G value from a likelihood-ratio genotype goodness-of-fit test will be identical when the pooled sample exhibits perfect HW equilibria. If the pooled sample is not in HW equilibrium, both allele and genotype goodness-of-fit tests can suffer from changes in type I error from the assumed α level (Schaid and Jacobsen 1999). The incorporation of a disequilibria term in the log-linear model prior to testing for concordant allele frequency or equilibria will have the effect of reducing this bias, similar to the influence of other corrections aimed at removing bias from HW disequilibrium (Schaid and Jacobsen 1999).

Our numerical analysis implies that the monogenic disequilibria coefficients from the log-linear model will be equal only when genotypes do not differ in their risk of disease. Different genetic etiologies (*e.g.*, multiplicative or additive genetic allele interactions) should therefore be detectable. Interestingly, the D coefficients in the affected and unaffected samples are identical when

additivity occurs in a biallelic system ($\omega_{AA} = 1$, $\omega_{Aa} = 0.5$, $\omega_{aa} = 0$). The further instances in which D of the two samples can be equal indicate that additive statistical models are inappropriate to assess groups for concordant equilibria.

Sparse data will cause an apparent increased type I error rate for the conventional allele and genotype goodness-of-fit test as well as the log-linear models presented here (because the results concerning the distribution of the test statistic are asymptotic). The solution for such sparse data analysis is the same—generating the null distribution of the test statistic by resampling from the expected tables with the constraint that the permuted table marginals are the same as that of the observed table.

One of the potential advantages of comparing disequilibria coefficients between groups is that examination of coefficients should provide insights into the relationship between genotype and phenotype (Hernandez and Weir 1989). For analyses involving the phase unknown two-locus model, consideration must be given to additional four di- and trigenic coefficients. While the biological basis for significance of such coefficients may not be straightforward it seems likely that biological meaning can be attributed to them (Weir and Cockerham 1989). For example, large protein complexes involving several different genes, or multiple copies of the same gene, may be candidates in which different combinations of alleles from the member proteins can impact on the functional attributes of such complexes.

A shortcoming of HW tests on a single group is that under some combinations of ω_{ij}, a group may experience high levels of selection and yet retain HW proportions ($M_{AA} = 1$). This occurs when the heterozygote and homozygote coefficients fulfill the relationship $\omega_{Aa}^2 = \omega_{AA}\omega_{aa}$ (Lewontin and Cockerham 1959), resulting in the appearance of HWE in the unaffected group. Similarly, the inverse situation ($s_{Aa}^2 = s_{AA}s_{aa}$) will also result in apparent HWE in the affected group. Under the latter condition, the fine scale mapping method of Nielsen *et al.* (1999) will fail. However, methods that utilize a reference sample will be informative when either the affected or unaffected group fulfills this condition, since the coefficients for the other group are not in accord with this relationship (*i.e.*, if $\omega_{Aa}^2 = \omega_{AA}\omega_{aa}$ then $s_{Aa}^2 \neq s_{AA}s_{aa}$). Thus, when $M_{AA} = 1$ for one group, $M_{AA} \neq 1$ for the other group. Additionally, fulfilling either the $\omega_{Aa}^2 = \omega_{AA}\omega_{aa}$ or the $s_{Aa}^2 = s_{AA}s_{aa}$ relationships requires that there be approximately at least a fourfold difference in ω or s, respectively, between the homozygotes. Therefore, the groups are also likely to exhibit both allele and genotype frequency differences.

A further potential shortcoming of HW-based tests for association would appear to be sensitivity to distortions arising from population admixture, since this can result in HW disequilibrium. If genetically differentiated populations that differ in disease incidence are inadvertently pooled in a disproportionate way between affecteds and unaffecteds, even unlinked markers can exhibit association. As for the standard allele and genotype goodness-of-fit tests, such potential confounding can be avoided by appropriate matching of affecteds and unaffecteds with regard to ethnic background. If affected and unaffected individuals are matched for ethnic background, and if there is no disease association, M_{AA} coefficients of the affected group, unaffected group, and total sample will all be the same, but not equal to 1.

As pointed out above, factors other than disease association can also lead to departure from HWE. Clearly, elimination of laboratory error as a potential source for departure from HWE is an essential first step. Excluding laboratory error, it is commonly assumed in genetic epidemiological studies that HW departure, manifest as excess homozygotes, in the random population of unaffecteds necessitates population admixture, and specific methods are employed to reduce the impact of this bias in evaluation of allele and genotypic frequencies between affecteds and unaffecteds (Chiano and Clayton 1998; Schaid and Jacobsen 1999). Yet available data for single-nucleotide polymorphisms do not support extensive genetic differentiation among the intensively studied populations from northwestern Europe (Cavalli-Sforza *et al.* 1994, p. 268; Goddard *et al.* 2000), for example, or even among the major ethnic groups (Barbujani *et al.* 1997). Moreover, as indicated above, under some combinations of penetrances the affected group may be in HWE while the unaffected group is in HW disequilibrium. Thus, a presumption of admixture should be avoided.

An important alternative to admixture is the operation of natural selection. For natural selection to cause detectable HW disequilibrium in a population the following are required: substantial fitness differences between genotypes, the selected genotype(s) be reasonably common, and the selective force also be reasonably common. Given these constraints, natural selection is not expected to be a frequent cause of HW disequilibrium. However, the classic example of the malarial resistance conferred by the β-globin allele $Hb\beta^S$ in African populations (Allison 1964), and HW disequilibrium where malaria is endemic, clearly demonstrates that the influence of natural selection on endemic human genetic variation is not just a theoretical possibility. Furthermore, by virtue of their involvement in regulating important biological functions, human candidate disease genes might be reasonably considered *a priori* to have a higher likelihood of being subject to natural selection than anonymous markers. Thus, selective origins for HW disequilibrium in random population unaffecteds should not be automatically dismissed.

Possible applications of the two-locus tests: The potential utility of testing for concordant equilibria in studies of affected and unaffected individuals is not restricted to single-locus comparisons. Many diseases may be polygenic, and epistatic genetic interactions, both

within and between loci, are likely to be important in the etiology of the disease phenotype. One consequence of this interlocus dependence can be disequilibria between the loci. Either of the phase-known or phase-unknown two-locus models may therefore be used to test for a role of epistatic interactions in genetic association studies.

There is also considerable value in comparing disequilibria coefficients between natural populations. In cases where evolutionary parameters are known to differ between population samples, a formal comparison of disequilibria coefficients would provide a valuable test of theoretical expectations. Additionally, comparing disequilibria coefficients may be used as an exploratory tool to assess whether differences exist between population samples.

The causes of genetic differentiation between wild populations will almost certainly be more complex than the genetic model we have outlined for epidemiological studies (Table 1). In comparing wild populations, the differential incidence of any evolutionary process that can cause departures from HW or linkage equilibrium is a candidate for detected genetic differentiation. The interpretation of genetic differences between populations will therefore require combining knowledge of theory with knowledge of population attributes.

Summary: The ease with which log-linear models can be modified to incorporate different terms has been illustrated here by our addition of terms to log-linear models of HW and linkage equilibrium. Increasingly complex data sets aimed at characterizing patterns of genetic differentiation using multiallelic loci or multiple single-nucleotide polymorphisms from multiple genes (Goddard *et al.* 2000) can also be readily accommodated by including additional terms (Zhang *et al.* 1990). The models we have presented enable population samples to be formally tested for concordant equilibria, providing a biologically intuitive framework for the examination of genetic differentiation. The extensive theory describing the effect of evolutionary processes on disequilibria coefficients can then serve as a rich backdrop from which to understand the nature of biological processes contributing to the genetic differentiation between samples.

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