Analysis of Multilocus Genetic Systems in Tecumseh, Michigan.
I. Definition of the Data Set and Tests for Goodness-of-Fit to Expectations Based on Gene, Gamete, and Single-Locus Phenotype Frequencies

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INTRODUCTION

Most population geneticists, until a few years ago, would not have argued with the assertion that selection is necessary to maintain alleles in a population in polymorphic frequencies. However, Kimura [1] and King and Jukes [2], using data on amino acid substitutions, proposed that much of the inherited protein variation we observe between and within species is not the result of selection, but rather is due to random drift of selectively neutral mutations. This "neutral hypothesis" derives from their interpretation of two observations. First, the high proportion (30%–40%) of the loci estimated to be polymorphic in natural populations of outbreeding organisms requires an excessive genetic load if balanced selection were responsible for the maintenance of the variation. Second, amino acid differences for most proteins which are observed among individuals within and between species appear to be functionally equivalent.

The alternative point of view, that most inherited variations are not simply random noise, states that the formulation of "load" has been unrealistic and that the failure to reject functional equivalence is a consequence of our inability to obtain proper measurements of the biological system. To answer this question, evidence to reject the neutral hypothesis is the prime requisite.

The meaning of such a large number of polymorphic loci to a population of organisms is no less a problem in our efforts to understand the human genome [3]. Although considerable work has been put forth to detect the operation of selection on inherited human biochemical variations (see [4] for review), no conclusion which is consistent among populations has been possible for any one polymorphic locus. The previous searches for evidence of selection can be partitioned into several categories (see [5]). Probably the most common approach has been to measure deviations from Hardy-Weinberg expectations. When the appropriate information is

available, differences between genotype or phenotype frequencies may be related to sex, age, or disease states to indicate possible correlation with differential fitness. When family data are available, segregation analysis has been used to detect evidence for selection [5–9]. However, one common objection to almost all such studies is that the analyses have been carried out on single loci.

It is well known that, with regard to overall fitness of an individual, the phenotype is the unit of selection. Each phenotype is the consequence of a complex interaction of the genotype with the environment. Furthermore, a genotype is not a collection of independent genes but is a set of spatially related and often interacting loci. Thus, any analysis which considers the linkage and interaction between loci should be more informative than one which treats the genotype as a set of discrete independent loci. Although Workman [10] and Franklin and Lewontin [11] have cited the need for such studies, very few bodies of data have been available for the proper analysis. The ABO-Rh relationship is one interaction which has been studied in depth (see [12] for a discussion) but is probably not representative of the typical multilocus effect. Data collected on 9,182 individuals of the Tecumseh Community Health Study provide one of the few opportunities to apply a multilocus analysis to the genetic variation in a human population. (A preliminary report by Reed [13] of an analysis of phenotype associations indicates a second body of human data which is appropriate for multilocus investigations.)

Ideally one would like to consider simultaneously all loci of the organisms which make up the population. However, there is a practical limitation as to the analysis which is possible. That is, the observed numbers in most genotypic classes when the number of loci is large would be too small for any statistical analysis to be meaningful. On the other hand, multiple two-locus analyses of variation in natural populations offer a sufficient first approximation of the extent of organization of the genotype, and sample sizes needed for the statistical analysis need not be unrealistically large.

Most multilocus studies have been theoretical considerations of two loci and have treated the interaction of selection and linkage as the primary forces in determining genotype frequencies. A few multilocus studies of natural or experimental populations have been reported. In Drosophila, Cannon [14] reported the analysis of a group of five linked loci. She found that although no deviation from Hardy-Weinberg occurred when single loci were considered, there were significant deviations for certain combinations when two loci at a time were analyzed. Mukai et al. [15] have recently reported that linkage disequilibrium could not be detected among pairs of four-enzyme loci but that nonrandom association of certain alleles and polymorphic chromosome inversions could be detected. The studies of Reed [13] and Shreffler et al. [16], using a contingency χ^2 analysis of two-locus phenotypes, indicate no consistent nonrandom associations among phenotypes of the blood-group systems of man. Turner [17] studied the MNSs system in an attempt to distinguish between overepistasis and underepistasis, the theory of which he had previously worked out [18–20]. He found that selection on the MNSs system showed overepistasis (i.e., double homozygotes are selected against more strongly

than single homozygotes). Workman [10] analyzed data on all possible pairs of loci of a small sample of Xavante Indians collected by Neel et al. [21]. The analysis failed to find any statistically significant deviations from random association of single-locus phenotypes. In general, the few multilocus analyses that have been carried out have not been more informative than single-locus analyses in identifying selection as a factor in determining genotype frequencies.

In this paper and the papers to follow we will report a detailed analysis of multilocus variation using data collected from the Tecumseh population. This first paper will describe the data, test for homogeneity of two-locus phenotype frequencies among various subdivisions of the total sample, and determine goodness-of-fit of these frequencies to Hardy-Weinberg expectations based on gene, gamete, and single-locus phenotype frequencies. Subsequent publications will present an analysis of gametic (or linkage) disequilibrium to estimate the correlations between alleles at different loci but in the same gamete.

MATERIALS AND METHODS

The blood, serum protein, and saliva systems utilized in this study have been described in previous reports [9, 16]. Analysis of 11 different genetic systems was performed as detailed below. The 11 genetic systems can be divided into six codominant genes (haptoglobin, Gc, MN, Ss, Rh-C, and Rh-E) and eight loci with dominance (ABO, P, Duffy, ABH secretor, Lewis secretor, Rh-D, Kidd, and Kell). (Kell is actually a codominant system but due to the rarity of KK genotypes, it was treated as a dominant system with little or no loss of information.) Except for the MNSs and Rh complexes, all loci are considered to be unlinked.

Blood and saliva samples were obtained from 9,182 Caucasoid individuals (approximately 90% of the population) of the community of Tecumseh, Michigan. Only individuals who had been tested for all 11 genetic systems are included in the study reported here. A total of 6,756 met this criterion. For the Kidd system two sera were used. Kidd type a sera was used on 5,545 individuals and Kidd type b sera on 1,211 individuals.

The 11 genetic systems were considered to contain 14 loci with 29 alleles. Including both the Kidd a and Kidd b systems, there are 104 possible two-locus combinations. Table 1 gives the genetic systems and loci analyzed in this study.

To test for homogeneity of the two-locus bivariate arrays among subdivisions of the population, the available data were stratified in the following manner: the 6,756 completely tested individuals designating the TOTAL sample were divided into two parts, KIN and NO KIN. The KIN group consisted of all people who had either a parent, child, or sibling in the study, and the NO KIN group consisted of all other individuals. The KIN group was further subdivided into parents (PARENT group) and their children (CHILD group). The difference between KIN and the sum of the PARENT and CHILD groups is that group of individuals who are related but had no offspring among the 6,756 individuals. The number of individuals in each of the five groups (TOTAL, NO KIN, KIN, PARENT, and CHILD) is shown in table 2. The PARENT and CHILD groups were not mutually exclusive since about 6% of the individuals were in both groups. For descriptive purposes, and for comparisons to be made later, the gene frequencies of the subsamples described in table 2 are presented in table 3.

A statistical analysis was applied to data representing each of the five groups. Within each group this analysis was performed for each of the 104 possible two-locus combinations. The analysis of each two-locus combination in each of the five groups consisted of three parts. First, gametic frequencies (from which gene frequencies were obtained) were estimated by a general maximum-likelihood program modified from MAXLIK [22]. Second,

TABLE 1

ALLELES AND PHENOTYPES OF 11 GENETIC SYSTEMS ANALYZED

System	Alleles	Phenotypes
Haptoglobin	Hp^1, Hp^2	Hp 1-1, Hp 1-2, Hp 2-2
Gc	Gc^1, Gc^2	Gc 1-1, Gc 1-2, Gc 2-2
MNSs	M, N S, s	MM, MN, NN SS, Ss, ss
Rh	C, c E, e D, d	CC, Cc, cc EE, Ee, ee D, dd
Duffy	Fy^a, Fy^b	Fy (a+), Fy (a—)
P	P^1, P^2	P1, P2
ABH secretion	Se, se	Secretor, nonsecretor
Lewis secretion	Le, le	LeS, nL
Kell	K, k	K, kk
Kidd	Jk^a, Jk^b	Jk (a+), Jk (a—) Jk (b+), Jk (b—)
ABO	A, B, O	A, B, AB, OO

χ^2 tests were used to detect heterogeneity of gene frequencies and bivariate phenotypic arrays among groups. These tests were run for each of the 104 possible two-locus combinations to determine if there were significant differences between the KIN and NO KIN samples of TOTAL and between the PARENT and CHILD subdivision of KIN. The homogeneity of gene frequencies was tested by $X^2 = (\hat{p}_1 - \hat{p}_2)^2 / [V(\hat{p}_1) + V(\hat{p}_2)]$, where $\hat{p}_1$ and $\hat{p}_2$ are the estimated gene frequencies (see table 3) of the two samples and $V(\hat{p}_1)$, $V(\hat{p}_2)$ are the estimated variances of $\hat{p}_1$ and $\hat{p}_2$, respectively (see [23]). This statistic, X^2, is distributed approximately as a χ^2 with one 1 df. The usual contingency χ^2 with $(r - 1)$ $(c - 1)$ df (see [24]), designated below as χ^2_H, was used to detect heterogeneity of the c two-locus phenotypes among r groups. The third aspect of the analysis of each two-locus combination consisted of a χ^2 test for goodness-of-fit to expected frequencies

TABLE 2

SAMPLE SIZES FOR GROUPS ANALYZED

	No. Two-Locus Combinations	No. Individuals in Each Combination in				
		TOTAL	NO KIN	KIN	PARENT	CHILD
All two-locus combinations except those involving Kidd a or Kidd b	78	6,756	985	5,771	2,351	2,837
Two-locus combinations involving Kidd a	13	5,545	825	4,720	1,918	2,335
Two-locus combinations involving Kidd b	13	1,211	160	1,051	433	502

TABLE 3

GENE FREQUENCIES FOR BLOOD-GROUP SYSTEMS

GENE	TOTAL (1)*	TOTAL (2)†	TOTAL (3)‡	NO KIN (1)	NO KIN (2)	NO KIN (3)	KIN (1)	KIN (2)	KIN (3)	PARENT (1)	PARENT (2)	PARENT (3)	CHILD (1)	CHILD (2)	CHILD (3)
Hp^1	.4139	.4116	.4244	.3959	.3927	.4125	.4170	.4149	.4262	.4113	.4087	.4226	.4194	.4162	.4226
Gc^1	.7149	.7175	.7031	.7142	.7188	.6906	.7150	.7172	.7050	.7176	.7161	.7240	.7092	.7137	.6882
M	.5500	.5497	.5516	.5543	.5612	.5187	.5493	.5476	.5566	.5442	.5409	.5589	.5558	.5559	.5587
S	.3207	.3231	.3100	.3193	.3248	.2906	.3210	.3228	.3130	.3224	.3240	.3152	.3211	.3225	.3147
C	.4115	.4114	.4120	.4193	.4109	.4625	.4102	.4115	.4043	.4117	.4147	.3984	.4138	.4152	.4073
E	.1613	.1618	.1589	.1766	.1812	.1531	.1587	.1584	.1598	.1610	.1593	.1686	.1570	.1565	.1593
D	.5819	.5804	.5885	.5919	.5851	.6292	.5801	.5796	.5826	.5860	.5846	.5922	.5840	.5834	.5860
Fy^a	.4315	.4334	.4231	.4116	.4194	.3725	.4350	.4358	.4312	.4232	.4205	.4354	.4500	.4532	.4354
P^1	.5289	.5300	.5243	.5263	.5290	.5126	.5294	.5301	.5261	.5356	.5348	.5390	.5344	.5419	.5010
Se	.4988	.5000	.4932	.5033	.5063	.4876	.4980	.4989	.4941	.5029	.5008	.5122	.4934	.4999	.4644
Le	.7539	.7495	.7755	.7637	.7612	.7764	.7522	.7474	.7754	.7611	.7562	.7848	.7481	.7414	.7813
K	.0378	.0401	.0272	.0399	.0421	.0285	.0374	.0398	.0270	.0375	.0396	.0281	.0384	.0415	.0242
Jk^a	...	.5117		...	.5063		...	.5126		...	.5221		...	.5085	
Jk^b			.4868			.4938			.4858			.4847			.5011
A	.2750	.2751	.2744	.2700	.2571	.3401	.2759	.2783	.2649	.2723	.2715	.2761	.2838	.2896	.2575
B	.0665	.0656	.0707	.0666	.0670	.0649	.0664	.0653	.0715	.0669	.0660	.0707	.0664	.0643	.0767
O	.6585	.6593	.6549	.6634	.6759	.5950	.6577	.6564	.6636	.6608	.6625	.6532	.6498	.6461	.6658

* = gene frequency when locus is combined with all but Kidd system.

† = gene frequency when designated locus is in combination with Kidd a.

‡ = gene frequency when designated locus is in combination with Kidd b.

within each group. One goodness-of-fit χ^2 (χ^2_G) was calculated with expected numbers based on gametic frequencies; a second (χ^2_T) was computed with expected numbers based on gene frequencies; and the third (χ^2_C) had expected numbers based on the product of the observed single-locus phenotype frequencies. To illustrate, within a group, consider the genotype *AABB* and let x_1 = estimated frequency of the *AB* gamete; p_A = estimated frequency of the *A* allele; p_B = estimated frequency of the *B* allele; f_{AA} = observed frequency of the *AA* genotype; and f_{BB} = observed frequency of the *BB* genotype. In a sample of N individuals, the expected number of *AABB* individuals is $x_1^2 N$ for χ^2_G, $p_A^2 p_B^2 N$ for χ^2_T, and $f_{AA}f_{BB}N$ for χ^2_C. Table 4 presents the number of two-locus

TABLE 4

DEGREES OF FREEDOM FOR χ^2'S CALCULATED FROM VARIOUS
PAIRWISE COMBINATIONS OF TWO-LOCUS SYSTEMS

| | | | No. PHENO-TYPIC CLASSES | DEGREES OF FREEDOM | | | |
SYSTEM 1	SYSTEM 2	No. COMBINATIONS	WITHIN EACH COMBINATION	χ^2_H	χ^2_G	χ^2_T	χ^2_C
Codominant	Codominant	15	9	8	5	6	4
Codominant	Dominant	48	6	5	2	3	2
Dominant	Dominant	27	4	3	0	1	1
ABO	Codominant	6	12	11	6	8	6
ABO	Dominant	8	8	7	2	4	3

combinations, the number of phenotypic classes in each combination, and the degrees of freedom for each χ^2.

In summary, for each two-locus combination: (1) gametic (and gene) frequencies were estimated for each group; (2) tests for homogeneity of KIN–NO KIN subdivision of TOTAL and of the PARENT-CHILD subdivision of KIN were carried out; and (3) the goodness-of-fit of the bivariate phenotype frequencies to expected numbers based on gene, gamete, and single-locus phenotypes frequencies were computed for each group.

RESULTS

Table 5 summarizes the analysis of homogeneity of two-locus phenotype frequencies among groups. The Hp-Kidd *a*, Ss-Rh-E, Rh-C–Rh-E, Rh-D–Rh-E, P–Rh-E, and Kell–Rh-E combinations of phenotypes varied significantly between the KIN and NO KIN groups (see χ^2_H column of KIN–NO KIN). The hypothesis of homogeneity was also rejected for seven combinations in the PARENT-CHILD comparison (table 5).

There are three orthogonal partitions of χ^2_H which may contribute to a significant heterogeneity of phenotype frequencies between groups. One component is due to the failure of the phenotype frequencies of the first locus to represent samples from the same population, the second component is due to heterogeneity at the second locus, and the third component is due to the failure of the interaction between the two loci to be the same in each group. Those components of χ^2_H of the

TABLE 5

LEVEL OF SIGNIFICANCE OF χ^2 TESTS FOR HETEROGENEITY OF PHENOTYPE FREQUENCIES AND GENE FREQUENCIES BETWEEN KIN AND NO KIN AND BETWEEN PARENT AND CHILD GROUPS

	KIN–NO KIN						PARENT-CHILD					
	χ^2_H (1)	Components of χ^2_H First Locus (2)	Second Locus (3)	Interaction (4)	Heterogeneity of Gene Frequencies First Locus (5)	Second Locus (6)	χ^2_H (7)	Components of χ^2_H First Locus (8)	Second Locus (9)	Interaction (10)	Heterogeneity of Gene Frequencies First Locus (11)	Second Locus (12)
COMBINATION	---	---	---	---	---	---	---	---	---	---	---	---
Hp-Kidd a	.05*	...	...	.05	...	...	...	.05	...	...	...	...
Ss-Rh-E	.05	...	.05	.05	...	...	...	...	...	...	...	...
Rh-C-Rh-E	.01	...	.05	.01	...	...	...	...	...	...	...	...
Rh-D-Rh-E	.01	...	.05	.05	...	...	...	...	...	...	...	...
P-Rh-E	.01	...	.05	.05	...	...	...	...	...	...	...	...
Kell-Rh-E	.05	...	.05	.05	...	...	...	...	...	...	...	...
ABH secretion-MN	...	...	...	.05	...	...	...	...	...	...	...	...
Ss-Kidd b	...	...	...	.05	...	...	...	...	...	...	...	...
Hp-Gc	...	...	...	...	...	...	.05	...	...	.05	...	...
Hp-Duffy	...	...	...	...	...	...	.05	...	.05	...	...	.05
P-Duffy	...	...	...	...	...	...	.05	...	.05	...	...	.05
ABH secretion-Duffy	...	...	...	...	...	...	.05	...	.05	...	...	.05
Lewis secretion-Duffy	...	...	...	...	...	...	.05	...	.05	...	...	.05
Kidd a-Duffy	...	...	...	...	...	...	.05	...	.05	...	...	.05
Lewis secretion-ABH secretion	...	...	...	...	...	...	.01	...	...	.01	...	...
ABO-Rh-E	...	...	.05	...	...	...	...	...	...	.05	...	...
Kidd a-Rh-E	...	...	...	...	...	.05	...	...	...	...	...	...
Kidd b-ABO	...	...	...	...	...	.05†	...	...	...	...	...	...

* Probability of observing a larger χ^2 given that the null hypothesis is true.

† Only the A allele is heterogeneous.

KIN–NO KIN comparison which are significant are indicated in columns 2, 3, and 4 of table 5. In only one case, Hp-Kidd a, was the significant difference attributable only to the interaction component. All others involved Rh-E where both a significant heterogeneity of phenotype frequencies at the E locus and a significant heterogeneity of interaction were observed. A similar partition of the significant PARENT-CHILD values of χ_H^2 (table 5) revealed that the Hp-Gc and ABH secretion–Lewis secretion combinations had significant heterogeneity of interaction, while the combinations involving Duffy all showed heterogeneity between groups for the phenotype frequencies at the Duffy locus.

It is possible that for cases in which the total homogeneity χ^2 (χ_H^2) is not significant, one (or more) components may be significant. We have included in table 5 those combinations in the KIN–NO KIN and PARENT-CHILD comparisons for which this situation obtains. A significant heterogeneity of interaction was detected for the ABH secretion–MN and Ss-Kidd b combinations in the KIN–NO KIN comparison and in the ABO–Rh-E combination in the PARENT-CHILD comparison. Heterogeneity of phenotype frequencies at the haptoglobin locus was detected in the PARENT-CHILD comparison.

Finally, the results of the test for heterogeneity of gene frequencies indicated that only the Rh-E, Duffy, and A (of ABO) loci were heterogeneous between groups (see table 5, cols. 6 and 12). Although the phenotypes of Rh-E in the KIN–NO KIN contrast were heterogeneous, the gene frequencies were not. The tests for heterogeneity of phenotype and gene frequencies for the Duffy system in the PARENT-CHILD contrast are correlated; hence they reflect only a difference in gene frequency.

The two-locus combinations that yielded significant goodness-of-fit χ^2's, that is, χ_T^2, χ_G^2, and χ_C^2, are presented in table 6. Within each group there are 104 possible goodness-of-fit values for χ_T^2 and χ_G^2. Since no goodness-of-fit test based on gametes (χ_G^2) was possible for the dominant-dominant two-locus combinations (e.g., Duffy-P, ABH secretion–Lewis secretion, Rh-D–Kell, etc.) because the degrees of freedom were exhausted in the estimation of gametic frequencies, only 77 χ_G^2 tests were applied.

We expect the MNSs and Rh combinations to give significant χ_T^2 and χ_C^2 since these systems do not represent independent loci. The remaining significant goodness-of-fit tests (at the .05 level of probability) for each χ^2 for each group are given in table 7. It is apparent that the number of significant tests deviates little from the number expected by chance (i.e., the number of tests $\times$ .05 equals the number of tests expected to be significant by chance alone). Among those tests which are significant, there is evidence to suggest that all are not simply Type I errors. For instance, in the NO KIN group, Rh-E occurs in three of four significant χ_G^2's, while in the CHILD group haptoglobin is involved in all three significant combinations when tested by χ_G^2. Also, in the CHILD group, haptoglobin and Kidd a systems are involved in three of seven significant χ_T^2's (excluding the MNSs and Rh combinations).

TABLE 6

SIGNIFICANCE LEVELS FOR GOODNESS-OF-FIT OF TWO-LOCUS PHENOTYPE FREQUENCIES TO
EXPECTATIONS BASED ON GENE FREQUENCIES (χ^2_T), GAMETE FREQUENCIES (χ^2_G) AND
SINGLE-LOCUS PHENOTYPE FREQUENCIES (χ^2_C), FOR FIVE GROUPS STUDIED

Combination	χ^2_T	χ^2_G	χ^2_C
Hp-Gc	C	c	C
MN–Rh-C	c	...	kC
Rh-D–Hp	c	c	...
Kidd *a*-Hp	k,C	k,c	kC
Kidd *b*-Hp	k	...	...
Kidd *a*-MN	t,c	...	t,k,C
Kidd *b*-Ss	k	...	k
Kidd *a*–Rh-C	c	...	k,c
P–Rh-E	N	N	n
Duffy–ABH secretion	T,k,C	...	T,k,C
P-Kidd *b*	p	...	p
ABH secretion–Lewis secretion	T,K,P	...	T,K,P
Lewis secretion–Kidd *a*	k,p	...	k,p
Rh-D–Gc	...	n	...
Kell-Ss	...	k	k
Kidd *b*–Rh-E	...	n	n
Gc-MN	...	...	p
ABH secretion-MN	...	...	c
Kidd *a*-Ss	...	...	t
ABH secretion–Rh-E	...	...	c
Kell–Rh-E	...	...	c
MN-Ss	TNKPC	...	TNKPC
Rh-C–Rh-E	TNKPC	n	TNKPC
Rh-D–Rh-E	TNKPC	...	TNKPC
Rh-D–Rh-C	TNKPC	...	TNKPC

NOTE.—T,t = TOTAL group; N,n = NO KIN group; K,k = KIN group; P,p = PARENT group; C,c = CHILD group. Uppercase letters indicate significance at the .01 level of probability; lowercase letters indicate significance at the .05 level.

TABLE 7

PERCENTAGE GOODNESS-OF-FIT χ^2 VALUES SIGNIFICANT AT .05 LEVEL OF PROBABILITY
FOR EACH OF FIVE GROUPS STUDIED

χ^2	GROUP				
	TOTAL	NO KIN	KIN	PARENT	CHILD
χ^2_T	3.0	1.0	6.0	3.0	6.0
χ^2_G	0.0	5.2	2.6	0.0	9.0
χ^2_C	4.0	2.0	9.0	4.0	9.0

A similar contingency χ^2 analysis (χ^2_C in table 6) has been considered by Shreffler et al. [16] for the total sample of 9,182. Certain differences are to be noted in comparison with the analysis presented here. Their study utilized all individuals who had been typed for a given pair of genetic systems, whereas in the present analysis an individual was included only if he were typed for all systems. Thus, comparing our TOTAL results with those of Shreffler et al., we find seven combinations that do not correspond. The Duffy-P combination, not significant in our analysis but significant in theirs, is most likely due to the children in the 0–3 age group (see [16] for discussion of effect of age on the P system). Most of these younger children were not typed for all systems and were therefore excluded from our TOTAL group. The lack of an ABO-ABH secretion interaction in the present analysis is probably a result of not distinguishing between the A_1 and A_2 alleles of the ABO system. The Lewis secretion–Kidd a combination was just barely significant at the .05 level of probability in the Shreffler et al. study ($\chi^2 = 3.84$, 1 df), and in our analysis the χ^2 was almost significant ($\chi^2 = 3.816$, 1 df). Finally, the Rh-Kell, Hp-Kell, Kidd-Kell, and Gc-Kell interactions found by Shreffler et al. were again probably a result of considering the separate genotypes of the Kell system (Kell a and Kell b).

Two combinations, Lewis secretion–ABH secretion and Duffy–ABH secretion, gave results which, while consistent with the Shreffler et al. analysis, indicate possible heterogeneity among groups. The Lewis secretion–ABH secretion combination gave significant deviations in TOTAL, KIN, and PARENT groups, and the Duffy–ABH secretion in TOTAL, KIN, and CHILD groups. This PARENT-CHILD heterogeneity is confirmed by χ^2_H (table 5).

DISCUSSION

The purpose of this paper was to describe the two-locus phenotypic arrays in different subdivisions of the Tecumseh population and to apply commonly used statistical tests to the data in an attempt to define deviations from Hardy-Weinberg expectations which may exist.

The tests for homogeneity of phenotype frequencies of the KIN–NO KIN subdivisions of TOTAL and the PARENT-CHILD subdivision of KIN indicated the groups differ for only a few two-locus combinations. Five of the six significant homogeneity tests of the KIN–NO KIN comparison had Rh-E as one of the two loci. In each of these there was a significant component due to heterogeneity among the Rh-E phenotypes and a significant component due to heterogeneity between groups for the interaction with the second system. A similar pattern appeared in the PARENT-CHILD comparison, where Duffy was implicated in five of seven significant homogeneity tests. Partitions of these indicated the heterogeneity in every case could be attributed to heterogeneity of gene frequencies for Duffy between the PARENT and CHILD groups. It is difficult to ascertain whether the significant heterogeneity between PARENT and CHILD groups for the interaction term in the Hp-Gc and Lewis secretion–ABH secretion combinations is real or fortuitous. On the basis of these comparisons, the subdivisions are probably homogeneous samples

with the exception of the differences attributable to the Rh-E and Duffy loci between KIN–NO KIN and PARENT-CHILD groups, respectively.

The analysis of the two-locus phenotypic arrays by goodness-of-fit to expectations based on gene, gamete, or single-locus phenotypes reveals that the two-locus phenotypes of children deviate more often from expected frequencies than do those of parents. The CHILD group yielded 11 more significant tests than the PARENT group and the KIN group yielded 10 more than the NO KIN group. These apparent differences between groups have no immediate explanation other than the possibility that the two-locus relationship may be modified by age. However, the direction of the deviation is not consistent with increasing exposure to selection with age.

A comparison of the results from the three tests, χ^2_T, χ^2_G, and χ^2_C (table 6), suggests to us an improvement in fit due to fitting gametic rather than gene or single-locus phenotype frequencies. This improvement may be attributable to fitting the gametic disequilibrium parameter to the data when one fits expectations based on gametic frequencies. A measure of genetic organization attributable to gametic disequilibrium is indicated which could not be detected by the standard goodness-of-fit based on gene frequencies or the contingency χ^2 analysis based on single-locus phenotype frequencies. The following paper will consider in detail the contribution of gametic disequilibrium to the nonrandom association of single-locus phenotypes.

SUMMARY

Analysis of two-locus phenotype frequencies by the conventional goodness-of-fit to Hardy-Weinberg expectation fails to reveal more than a few combinations which might indicate the presence of selection for multilocus phenotypes. As is often the case, in view of the number of tests generated, no clear indictment of nonrandom forces is possible. The analysis presented illustrates the utility of contrasting alternative statistical procedures as a methodology for identifying contributions of specific effects to the nonrandom distributions of genes in genotypes.

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