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HLA and human mate choice: tests on Japanese couples

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Abstract House mice are apparently more likely to mate with individuals dissimilar to themselves at MHC (major histocompatibility complex) loci than with similar individuals. Such negative assortative mating is thought to be mediated by olfaction. Recently, it has been suggested that human mate choice may be affected by HLA (human leukocyte antigen; MHC in humans), based on the finding that women prefer the odor of men dissimilar to themselves at HLA loci to that of HLA-similar men. If these odor preferences are indeed an important criterion of mate choice in humans, actual marriages may show negative assortment with respect to HLA. In this paper, we compared the observed similarity between spouses at HLA loci with the expected similarity under random mating, for about 150 couples from 6 prefectures in the Tohoku region of Japan, and for about 300 couples from 16 prefectures all over Japan. For statistical tests, we used empirical distributions of goodness-of-fit statistics, X^2 and G, obtained by Monte Carlo methods, because these statistics may not follow the chi-square distribution. Tests for each sample as a whole and for each prefecture rule out strong disassortative mating at the HLA-A and HLA-C loci.

Keywords: disassortative mating, mating preference, MHC, Monte Carlo method

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Introduction

MHC (major histocompatibility complex), known in humans as HLA (human leukocyte antigen), plays an important role in the immune system (Klein, 1986). The principal function of MHC molecules is to present antigens to T cells to initiate an immune response against nonself. The high degree of polymorphism in MHC genes is thought to be maintained by balancing selection such as overdominant or frequency-dependent selection from pathogens, and/or mating preferences (Hedrick, 1994).

Mate choice in house mice (*Mus musculus domesticus*) is apparently influenced by MHC. Many studies have been published showing that mice are more likely to mate with individuals dissimilar to themselves at MHC loci than with similar individuals (Yamazaki and others, 1976; Egid and Brown, 1989; Potts and others, 1991; reviewed in Penn and Potts, 1999; but see discussion). More precisely, mice appear to imprint negatively on the MHC identities of the individuals by, or with, whom they are raised, who under normal circumstances would be extended family (Yamazaki and others, 1988). Such negative assortative (or disassortative) mating with respect to MHC is thought to be mediated by olfaction (Yamazaki and others, 1979; reviewed in Penn and Potts, 1998).

There are three hypotheses proposed to explain the function of MHC-dependent mating preferences (Penn and Potts, 1999). First, offspring of individuals that mate disassortatively will tend to be heterozygous at MHC loci. If MHC-heterozygotes have a higher fitness than homozygotes (Black and Salzano, 1981; Thursz and others, 1997; Carrington and others, 1999), these individuals may have the advantage of having a greater number of surviving offspring. Second, offspring from MHC-disassortative mating are likely to be dissimilar from their parents at MHC loci. If MHC diversity is maintained by a coevolutionary arms race between hosts and parasites, this may provide a “moving target” to parasites that adapt to their host’s genotype. Third, since MHC genes are highly polymorphic, two individuals similar to each other at MHC loci are likely to be relatives (reviewed in Brown and Eklund, 1994). Hence, negative assortative mating with respect to MHC leads to the avoidance of inbreeding, which is often deleterious (for human inbreeding see Adams and Neel, 1967; Seemanová, 1971; Bittles and Neel, 1994).

Wedekind and others (1995) showed experimentally that human odor preference is associated with HLA dissimilarity. They examined preferences of female university students for the odor of T-shirts worn by male university students who were similar or dissimilar to themselves at HLA loci (HLA-A, -B, and -DR). The females in the study found the odor of the T-shirts worn by the HLA-dissimilar males to be more pleasant and sexy than the odor of the T-shirts worn by the HLA-similar males. Wedekind and others also showed that the odor of HLA-dissimilar males reminded females more often of their current or previous mates than did the odor of HLA-similar males. A more recent study using a somewhat different experimental design basically supported these results (Wedekind and Füri, 1997).

If these odor preferences are reflected in actual marriages, negative assortative mating with respect to HLA may be detected by examining the association of HLA types between spouses. Two recent attempts to detect such negative assortative mating have reached opposite conclusions. Ober and others

(1997) studied 411 couples from the Hutterite population, a North American reproductive isolate of European ancestry. Evaluating five-locus haplotypes (HLA-A, -C, -B, -DR, and -DQ), they found fewer matches for HLA haplotypes between spouses than expected from random mating and concluded that negative assortative mating was actually occurring in the Hutterite population. Hedrick and Black (1997) examined the number of shared HLA-A and HLA-B alleles between spouses in 194 South Amerindians couples from the lower Amazon basin. In contrast to Ober and others they found no evidence for non-random mating, a result consistent with some earlier studies (Pollack and others, 1982; Rosenberg and others, 1983; Jin and others, 1995).

In this paper, we examine the similarity between spouses at HLA loci in Japanese couples and test for random mating with respect to HLA. The data analyzed here are from two distinct family studies. In one study, in order to determine the appropriate size of an unrelated donor pool for HLA-matched platelet transfusion and bone marrow transplantation, five-locus haplotypes were determined by serology for about 150 married couples from 6 prefectures in the Tohoku region (northeastern part of the main island) of Japan (Takahashi and others, 1992). This sample will be called “Tohoku” in this paper. Subjects were in principle limited to individuals who had been born in the Tohoku region, and no consanguineous marriages were observed. In the other study, which was for the 8th Japan HLA workshop, four-locus haplotypes were determined by serology for about 300 married couples from 16 prefectures all over Japan (see Fujii and others, 1983). This sample is referred to as “8JW” in the following. Unfortunately, because these samples were collected originally to evaluate the variability of HLA-types in Japanese, information that would be useful for a test of mate choice is not included (e.g. exact birthplaces of the subjects, whether the marriages had been arranged or not; see Discussion).

Methods

Marital associations at HLA loci were examined for each individual locus. First, the couples were classified into three categories according to the number of shared alleles, that is, matings that share no alleles (e.g. A1A2×A3A11), one allele (e.g. A1A2×A1A3, A1A2×A1A1), or two alleles (e.g. A1A2×A1A2, A1A1×A1A1). Only couples completely typed for the relevant locus were included. Second, the number of couples in each of the three categories expected from random mating was calculated based on observed male and female genotype frequencies. Finally, the observed and expected numbers were compared.

In order to test for random mating, we computed two goodness-of-fit statistics, X^2 and G, from the observed and expected numbers (see Sokal and Rohlf, 1995, pp. 685-793). The distributions of X^2 and G can be approximated by a chi-square distribution when sample size is large, and the degrees of freedom would be 2 if the expected numbers were based on an extrinsic hypothesis, i.e. a hypothesis external to the data. In this case, however, the expected numbers are computed from male and female genotype

frequencies that have been estimated from the sample. Hence, the distributions of X^2 and G cannot a priori be assumed to follow the chi-square distribution with 2 degrees of freedom (see Jin and others, 1995).

Empirical distributions of X^2 and G under the null hypothesis of random mating were obtained by Monte Carlo simulations. (Pseudo-random numbers were generated by the algorithm described in Schrage (1979).) The procedure described below was repeated 2000 times. (1) A sample of couples whose size was the same as the *actual* sample was generated by the random pairing of male and female genotypes at their observed frequencies. (2) In this *simulated* sample the numbers of couples that share no alleles, one allele, and two alleles were counted. (3) The expected numbers for the simulated sample were calculated based on male and female genotype frequencies in that sample. (4) X^2 and G were computed from the observed and expected numbers in the simulated sample. Empirical P-values for goodness-of-fit tests were obtained as the proportions of X^2- or G-values in the 2000 simulations that exceeded those values calculated for the actual sample. The tests were performed both for the Tohoku and 8JW samples as a whole and for each prefecture separately. In addition, we conducted overall tests of significance by combining probabilities from tests of random mating for each prefecture (Fisher, 1954; see Sokal and Rohlf, 1995, pp. 794-797). (It is not possible to combine probabilities across loci since they are not independent.)

Marital associations were also tested with respect to haplotypes. The tests were conducted by two distinct methods. In “method 1,” each haplotype was treated as an “allele” and the couples were classified into three categories according to the number of shared haplotypes as in the analyses for each locus. In “method 2,” each haplotype was no longer considered as an “allele” and the couples were classified into eleven (for Tohoku), or nine (for 8JW) categories according to the number of shared alleles. In both of the methods, expected numbers were calculated based on observed five- (for Tohoku), or four-locus (for 8JW) genotype frequencies and the deviations from the expectations were tested using Monte Carlo methods, which are essentially the same as in the tests for each locus.

We also examined geographical variation in genotype frequencies. To this end, genotype frequencies were compared among prefectures for each locus and for haplotypes. The comparisons were made for each sex, and for both sexes pooled. For each comparison, G for heterogeneity was computed to test the null hypothesis that the genotype frequencies do not differ among the prefectures. The tests were based on empirical distributions of G obtained by Monte Carlo methods, because the expectations were too small to permit approximation by a chi-square distribution. The procedure used is similar to the one described above; (1) A random sample of individuals of the same size as the actual sample was generated based on the genotype frequencies in the actual sample as a whole. (2) The simulated sample was partitioned into subsamples corresponding to the prefectures in the actual sample. (3) G-value for heterogeneity was computed for the simulated sample. This procedure was repeated 2000 times to obtain an empirical distribution of G. An empirical P-value was obtained as the proportion of G-values in the 2000 trials that exceeded the value derived from the actual sample.

Finally, we estimated the statistical power of our tests for random mating. To do this, a simulated

sample of couples was created for each test according to the model of disassortative mating described below. We tentatively formed each couple by the random pairing of male and female genotypes at their frequencies. Then the couple was accepted with probability $1 - [k/(2m)]s$, where k denotes the number of shared alleles, m is the number of relevant loci, and s is a measure of the strength of assortment ($0 \leq s \leq 1$). Otherwise, the couple was rejected and another one was drawn (hence the probability of rejection is assumed proportionate to the number of shared alleles standardized by the maximum number). Note that in terms of the tests for each locus and for haplotypes with method 1, the probabilities of acceptance reduce to 1, $1 - s/2$, and $1 - s$, for couples that share no alleles, one allele, and two alleles, respectively (Hedrick and Black, 1997). This procedure was repeated to obtain a simulated sample of couples of the same size as the actual one. The expected numbers were calculated as before by multiplying male and female genotype frequencies. Empirical distributions of X^2 and G under the alternative hypothesis of negative assortative mating were obtained from 2000 simulations and then compared to those for the null hypothesis of random mating. As an example, Fig. 1 shows the empirical distributions of G for the HLA-A locus in the Tohoku sample as a whole. 39% of the 2000 values for the alternative hypothesis with $s = 0.5$ exceed the 5% critical value of the null hypothesis, 5.14.

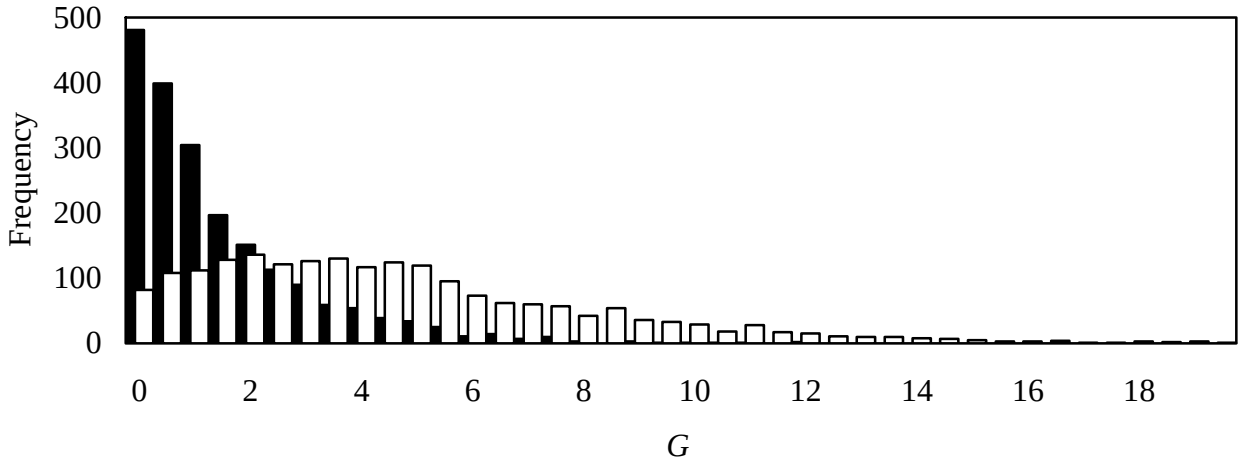

Figure 1. Empirical distributions of G obtained for the HLA-A locus in the Tohoku sample as a whole. The filled bars represent the empirical distribution under the null hypothesis and the empty bars are that of the alternative hypothesis ($s = 0.5$). The ith bar (starting from the left) represents the number of G-values that fall within the range, $0.5(i - 1) \leq G < 0.5i$, of 2000 simulated values. G-values greater than 20 are not shown (6 cases).

The statistical power of each test was estimated as the fraction of X^2- or G-values under the alternative hypothesis that exceeded the 5% critical value of the null hypothesis. The tests for each prefecture separately have low power. To increase power, we combined probabilities across prefectures (Fisher, 1954). In what follows, results are stated in terms of the G-statistic, because in our tests G-tests always had higher power than the X^2 counterparts, and our conclusions are the same whether X^2 or G are

used.

Results

Tests of Random Mating for Each Sample as a Whole

In the Tohoku sample, there were 9 alleles at the HLA-A locus, 8 alleles (including "blank") at the HLA-C locus, 22 alleles at the HLA-B locus, 13 alleles at the HLA-DR locus, and 6 alleles at the HLA-DQ locus. Five-locus haplotypes numbered 326. Observed and expected numbers of matings that share HLA alleles (or haplotypes for "method 1") are shown in the upper part of Table 1. Note that sample sizes differ according to the locus/loci analyzed because only couples completely typed for the locus/loci are included. The expectations were calculated assuming random mating with male and female genotype frequencies in the sample as a whole. For each locus and five-locus haplotypes, a statistical test was conducted based on an empirical distribution of *G* (see Methods). Only for the five-locus haplotypes was a statistically significant deviation from random mating observed ($P < 0.05$). However, observed mean number of shared haplotypes was greater than expected, which is contrary to what would be predicted by negative assortative mating. Furthermore, in the analyses for each locus, observed mean numbers of shared alleles were greater than expected for all of the loci analyzed, although none of the deviations were statistically significant.

Table 1. Number of matings that share HLA alleles (or haplotypes) in Tohoku and 8JW. MNSA stands for mean number of shared alleles, [number of matings that share one allele + number of matings that share two alleles × 2] / [total number of matings]. Numbers in parentheses are expectations under random mating. *N* is total number of matings. Each haplotype is considered as an "allele" (method 1). *$P < 0.05$ based on 2000 simulations.

Sample	Locus	Number of matings that share: no alleles		one allele		two alleles		*N*	MNSA		*G*
Tohoku	A	56	(58.75)	87	(82.06)	11	(13.19)	154	0.71	(0.70)	0.81
	C	58	(62.73)	84	(79.12)	11	(11.16)	153	0.69	(0.66)	0.66
	B	109	(111.95)	42	(40.16)	3	(1.89)	154	0.31	(0.29)	0.72
	DR	87	(95.53)	59	(52.07)	5	(3.40)	151	0.46	(0.39)	2.33
	DQ	47	(53.24)	87	(81.21)	17	(16.55)	151	0.80	(0.76)	1.18
	Haplotype	138	(141.84)	9	(6.07)	1	(0.08)	148	0.07	(0.04)	4.52*
8JW	A	114	(107.67)	148	(158.11)	29	(25.21)	291	0.71	(0.72)	1.57
	C	95	(96.37)	163	(153.67)	24	(31.96)	282	0.75	(0.77)	2.74
	B	206	(203.77)	72	(74.77)	4	(3.46)	282	0.28	(0.29)	0.21
	DR	95	(98.37)	78	(77.95)	11	(7.68)	184	0.54	(0.51)	1.38
	Haplotype	131	(133.96)	12	(8.92)	0	(0.12)	143	0.08	(0.06)	1.26

In the 8JW sample, there were 8 alleles at the HLA-A locus, 7 alleles (including "blank") at the HLA-C locus, 20 alleles at the HLA-B locus, and 12 alleles at the HLA-DR locus. Four-locus haplotypes numbered 274. Observed and expected numbers of matings that share HLA alleles (or haplotypes for

method 1) are shown in the lower part of Table 1. Statistical tests based on empirical distributions showed no evidence for non-random mating at any of the loci or for the four-locus haplotypes.

For most of these tests, the empirical values of *G* were biased toward smaller values compared to the chi-square distribution with 2 degrees of freedom. Hence, the tests would be conservative if these statistics were assumed to follow the chi-square distribution, which is consistent with earlier work by Jin and others (1995). The test with respect to the HLA-A locus in Tohoku, for example, *G*-values greater than the critical value of the chi-square test at the 5% level, i.e. 5.99, were observed in 54 among 2000 simulations (2.7%), suggesting that a test based on the chi-square distribution would be conservative. In sum, the chi-square distribution with 2 degrees of freedom is not unacceptable as an approximation, but an empirical distribution is preferable when circumstances permit.

Table 2 shows the results of complementary analyses with respect to haplotypes, i.e. "method 2." Observed and expected numbers of matings that share HLA alleles are shown. Neither the Tohoku nor 8JW samples showed a significant deviation from random mating.

Table 2. Number of matings that share HLA alleles in Tohoku and 8JW. Numbers in parentheses are expectations under random mating with respect to haplotypes. *N* is total number of matings. Each of the haplotypes consists of five (for Tohoku), or four (for 8JW) alleles (method 2). $P > 0.05$ in both samples based on 2000 simulations.

Number of shared alleles	Number of matings			
	Tohoku		8JW	
0	7	(9.25)	8	(11.24)
1	20	(24.34)	31	(34.10)
2	44	(34.44)	50	(41.80)
3	26	(33.53)	24	(30.41)
4	23	(23.40)	18	(17.22)
5	17	(14.35)	10	(6.38)
6	5	(6.06)	2	(1.43)
7	4	(1.95)	0	(0.30)
8	1	(0.51)	0	(0.12)
9	0	(0.07)	-	-
10	1	(0.08)	-	-
N	148		143	
G	11.71		7.14	
P	0.20		0.42	
Mean number of shared alleles	2.97 (2.80)		2.36 (2.25)	

Tests for Heterogeneity in Genotype Frequencies among Prefectures

So far, we have been testing the null hypothesis that random mating with respect to HLA occurs in the

Tohoku and 8JW samples as a whole. However, this may be misleading if the areas from which these samples were drawn in fact had a population structure so that genotype frequencies were different among subpopulations. In this case, even if people mate at random within each subpopulation, our analysis may suggest *positive* assortative mating. More to the point, disassortative mating at the subpopulation level may be masked. The failure to detect negative assortative mating and the finding of positive assortative mating described above might be explained by this effect. To see if regional differences in genotype frequencies exist we tested for heterogeneity in genotype frequencies among prefectures.

Differences in genotype frequencies among prefectures were detected in the Tohoku sample for HLA-B ($P < 0.01$ for each sex and for both sexes pooled), -DR ($0.01 < P < 0.05$ for males; $P < 0.01$ for females and for both sexes pooled), and five-locus haplotypes ($P < 0.01$ for each sex and for both sexes pooled), and in the 8JW sample, for HLA-A ($0.01 < P < 0.05$ for males; n.s. for females and for both sexes pooled), -B ($P < 0.01$ for each sex and for both sexes pooled), -DR ($P < 0.01$ for females; n.s. for males and for both sexes pooled), and four-locus haplotypes ($P < 0.01$ for each sex and for both sexes pooled). (see Methods for statistical tests.) Hence, population structure cannot be ignored in either sample.

Tests of Random Mating for Each Prefecture

To minimize the effect of subpopulation structure as mentioned above, tests of random mating were performed for each prefecture. The procedure was the same as the analyses for each sample as a whole except for smaller sample sizes (mean = 25.3, s.d. = 5.8 for Tohoku prefectures; mean = 15.6, s.d. = 11.6 for 8JW prefectures).

With analyses for each locus and for haplotypes in the same way as before, we found fourteen cases of non-random mating in a total of 132 tests ($P < 0.05$; the upper part of Table 3). Seven of the statistically significant outcomes were with respect to individual loci (Table 4); the deviations were toward negative assortment at the HLA-A and -C loci and toward positive assortment at the HLA-B and -DR loci. The other seven were with respect to haplotypes, and all the deviations were toward positive assortment (data not shown). Combining the probabilities from the tests for each prefecture, we found overall deviations from random mating with respect to the HLA-B locus and haplotypes using method 2 (the lower part of Table 3). In both cases, the observed mean numbers of shared alleles were, however, not different from the expected number under random mating. This is because both (nonsignificant) negative and (significant) positive assortative mating were observed within prefectures and the deviations cancelled out.

Table 3. Empirical *P*-values for the tests of random mating for each prefecture and overall tests of significance. MNSA stands for mean number of shared alleles. Numbers in parentheses are expectations under random mating. $^{**}P < 0.01$ for overall tests.

		Locus					Haplotype	
Sample	Prefecture	A	C	B	DR	DQ	Method 1	Method 2
Tohoku	Aomori	0.8895	0.6300	0.3680	0.3915	0.5810	0.6495	0.0300
	Akita	0.5015	0.4220	0.5610	0.2315	0.1060	0.8300	0.3480
	Iwate	0.6065	0.8155	0.7890	0.0825	0.2950	0.4895	0.6335
	Miyagi	0.5030	0.3265	0.8135	0.2330	0.6080	0.4830	0.7340
	Yamagata	0.8030	0.0675	0.2570	0.8405	0.2870	0.0190	0.1115
	Fukushima	0.4215	0.4125	0.4195	0.9375	0.3885	0.8585	0.3435
8JW	Hokkaido	0.9030	0.8045	0.5890	0.4520	-	0.3965	0.0935
	Miyagi	0.1010	0.0210	0.0045	0.0240	-	0.0130	0.1235
	Chiba	0.1450	0.2200	0.3595	0.6830	-	1.0000	0.1725
	Tokyo	0.9195	0.0475	0.5595	0.3350	-	0.5340	0.6715
	Saitama	0.1110	0.6210	0.5715	0.3260	-	0.3645	0.1880
	Kanagawa	0.5925	0.5950	0.7785	0.7285	-	0.8375	0.0365
	Shizuoka	0.2545	1.0000	-	0.2480	-	-	-
	Aichi	0.6080	0.6305	0.5655	0.9295	-	0.6760	0.0205
	Shiga	0.4215	0.1240	0.0060	0.2490	-	1.0000	0.2675
	Kyoto	0.0935	0.9080	0.5450	0.0585	-	1.0000	0.0170
	Osaka	0.2170	0.2175	0.1555	0.7795	-	0.0055	0.4660
	Hyogo	0.0250	0.5360	0.0640	0.4180	-	0.1870	0.1030
	Hiroshima	0.8200	0.6125	0.4005	0.0590	-	0.3915	0.3780
	Ehime	0.9405	0.8545	0.5940	-	-	-	-
	Fukuoka	0.1760	0.2990	0.0760	0.9390	-	0.0550	0.3955
	Nagasaki	0.4965	0.5390	0.0030	0.2560		1.0000	0.2480
Overall Tests								
Tohoku	$-2\Sigma \ln P$	6.16	12.46	8.50	13.18	13.40	12.35	17.18
	df	12	12	12	12	12	12	12
	MNSA	0.71	0.69	0.31	0.46	0.80	0.07	2.97
		(0.71)	(0.67)	(0.29)	(0.40)	(0.76)	(0.05)	(2.83)
8JW	$-2\Sigma \ln P$	40.29	33.81	58.14**	37.02	-	36.38	54.42**
	df	32	32	30	30	-	28	28
	MNSA	0.71	0.75	0.28	0.54	-	0.08	2.36
		(0.71)	(0.77)	(0.28)	(0.51)	-	(0.06)	(2.23)
Total	$-2\Sigma \ln P$	46.45	46.27	66.64**	50.20	-	48.73	71.60**
	df	44	44	42	42	-	40	40
	MNSA	0.71	0.73	0.29	0.50	-	0.08	2.67
		(0.71)	(0.74)	(0.28)	(0.46)	-	(0.05)	(2.54)

Table 4. Number of matings that share HLA alleles in the prefectures in which significant deviations from random mating were found. MNSA stands for mean number of shared alleles. Numbers in parentheses are expectations under random mating. *N* is total number of matings.

Locus	Sample	Prefecture	Number of matings that share						*N*	MNSA	
			no alleles		one allele		two alleles				
A	8JW	Hyogo	8	(4.36)	6	(8.50)	0	(1.14)	14	0.43	(0.77)
C	8JW	Miyagi	7	(3.80)	3	(5.00)	0	(1.20)	10	0.30	(0.74)
C	8JW	Tokyo	8	(13.61)	26	(18.86)	2	(3.53)	36	0.24	(0.29)
B	8JW	Miyagi	4	(7.11)	5	(1.89)	0	(0)	9	0.56	(0.21)
B	8JW	Shiga	5	(4.33)	0	(1.50)	1	(0.17)	6	0.33	(0.31)
B	8JW	Nagasaki	2	(6.33)	6	(2.33)	1	(0.33)	9	0.89	(0.33)
DR	8JW	Miyagi	2	(4.75)	6	(3.25)	0	(0)	8	0.75	(0.41)

Estimate of Statistical Power

Table 5 shows the estimated power of the tests for random mating. Values are given for each locus and for haplotypes and for the Tohoku and 8JW samples as a whole. A minority of the tests possessed a power high enough (i.e. greater than 0.90) to detect negative assortative mating with an *s*-value (see Methods) of 0.6 or larger. For example, the test for random mating at the HLA-C locus in the 8JW sample as a whole, which is applicable because the test for heterogeneity is nonsignificant, has a power of 0.91. It is unlikely, however, that the influence of HLA similarity on actual marriages as measured by *s* is as large as 0.6.

The tests of random mating conducted on each prefecture had, on the other hand, much lower power than those conducted on each sample as a whole. Few of these tests were powerful enough to detect an *s*-value even as large as 1.0. The power increased when the probabilities were combined though they were still lower than those of the tests for each sample as a whole (Table 6).

Summary of the Results

HLA-A: The null hypothesis of random mating was not rejected (see Table 1 and the lower part of Table 3). The effect of population structure can likely be ignored, since the test for heterogeneity is significant only for 8JW males at the 5% level. The power is relatively high (see Table 5, 6). This provides evidence against strong disassortative mating ($s > 0.6$) at this locus.

HLA-C: The null hypothesis was not rejected (see Table 1 and the lower part of Table 3). Population structure was not detected and the power is relatively high (see Table 5, 6). Hence strong disassortative mating ($s > 0.6$) is not supported with respect to this locus.

HLA-B: The heterogeneity tests were significant for both of samples and we suspect that population structure exists even within prefectures (see Table 4). Alternatively, the significant deviations from random mating within some of the prefectures may reflect positive assortative or consanguineous marriages (but see below). Hence no conclusions can be drawn for this locus.

Table 5. Estimated power of tests for random mating in Tohoku and 8JW. Estimated values are obtained for the *G*-tests at 5% level.

Sample	Locus / Haplotype	*s*-value					
		0.5	0.6	0.7	0.8	0.9	1.0
Tohoku	A	0.39	0.60	0.82	0.96	1.00	1.00
	C	0.41	0.62	0.83	0.96	1.00	1.00
	B	0.25	0.38	0.53	0.71	0.85	0.95
	DR	0.30	0.45	0.62	0.78	0.93	1.00
	DQ	0.44	0.65	0.86	0.97	1.00	1.00
	Haplotype (Method 1)	0.09	0.09	0.10	0.12	0.15	0.18
	Haplotype (Method 2)	0.06	0.08	0.08	0.12	0.17	0.26
8JW	A	0.68	0.90	0.99	1.00	1.00	1.00
	C	0.71	0.91	0.99	1.00	1.00	1.00
	B	0.49	0.68	0.85	0.95	0.99	1.00
	DR	0.44	0.64	0.83	0.95	1.00	1.00
	Haplotype (Method 1)	0.09	0.09	0.10	0.16	0.20	0.24
	Haplotype (Method 2)	0.07	0.09	0.11	0.16	0.21	0.29

HLA-DR: Non-random mating was not detected (see Table 1 and the lower part of Table 3). However, no conclusions can be drawn for this locus because population structure was indicated and the statistical power is low (see Table 5, 6).

HLA-DQ: The 8JW sample does not include data on this locus. The null hypothesis was not rejected (see Table 1, 3). There is no evidence of population structure and the power is relatively high (see Table 5, 6). We can, therefore, at least exclude the possibility of extremely strong disassortative mating ($s > 0.8$) occurring with respect to this locus in Tohoku.

Haplotypes: Since population structure was detected and the statistical power is very low (see Table 5, 6), no conclusions can be drawn.

Discussion

Negative assortative mating with respect to HLA was not detected in Japanese with the tests of random mating for each locus and for haplotypes. The results were essentially the same whether the tests were done on the Tohoku and 8JW samples as a whole or on each of the prefectures. In addition, differences in genotype frequencies at the HLA loci among prefectures were detected by the heterogeneity tests in both of the samples.

Table 6. Estimated power of overall tests for random mating for each prefecture. Estimated values are obtained for the *G*-tests at 5% level.

Sample	Locus / Haplotype	df	*s*-value			
			0.7	0.8	0.9	1.0
Tohoku	A	12	0.42	0.66	0.89	1.00
	C	12	0.45	0.67	0.88	1.00
	B	12	0.20	0.27	0.39	0.51
	DR	12	0.27	0.38	0.53	0.70
	DQ	12	0.50	0.76	0.95	1.00
	Haplotype (Method 1)	12	0.04	0.05	0.05	0.06
	Haplotype (Method 2)	12	0.05	0.05	0.05	0.06
8JW	A	32	0.41	0.66	0.92	1.00
	C	32	0.50	0.81	0.98	1.00
	B	30	0.22	0.33	0.46	0.64
	DR	30	0.11	0.18	0.29	0.45
	Haplotype (Method 1)	28	< 0.01	< 0.01	< 0.01	< 0.01
	Haplotype (Method 2)	28	0.02	0.02	0.02	0.02
Total	A	44	0.62	0.89	0.99	1.00
	C	44	0.71	0.95	1.00	1.00
	B	42	0.30	0.45	0.63	0.81
	DR	42	0.23	0.40	0.58	0.80
	Haplotype (Method 1)	40	< 0.01	< 0.01	< 0.01	< 0.01
	Haplotype (Method 2)	40	0.02	0.03	0.02	0.03

Wedekind and others (1995) and Wedekind and Füri (1997) found that people prefer the odor of a person who is dissimilar to themselves at HLA loci to the odor of a person similar to themselves and this suggests that their mate choice may be affected by such odor preferences. On this view, spouses should be more dissimilar to each other at HLA loci than expected from random mating. In the present study, however, we did not find any evidence for the hypothesis. This result suggests that the human odor preference claimed by Wedekind and his colleagues is not reflected in actual marriages at least in Japanese today. Alternatively, it is also possible that negative assortative mating is in fact occurring in Japanese, but the statistical power of our analyses was too weak to detect it (Tables 5, 6). In relation to the latter case, it should be noted that the influence of HLA on human mate choice is unlikely to be large because people use many criteria other than HLA similarity when they choose their mates (e.g., Buss, 1989). Our samples may be too small to detect such a small effect (for further discussion see Hedrick and Black, 1997).

In the tests of random mating for each sample as a whole, a significant deviation from random mating was observed only for five-locus haplotypes in Tohoku (method 1). Moreover, contrary to expectations, the deviation was toward positive assortative mating. This result may be due to the effect of population structure, which was not taken into account in this particular test, a contention which is

supported by the results of the heterogeneity tests.

To take the population structure into account, we should have calculated the expected number of shared alleles for each couple on the basis of the genotype frequencies in the prefectures where each of the spouses had been born. In fact, however, this was not possible due to lack of information on the birthplaces of the subjects. Instead of this, we tested random mating for each prefecture separately to minimize the effect of population structure. In this case, since each sample is divided into smaller subsamples the statistical power is reduced though the effect of population structure may be minimized. Although we found 14 cases of non-random mating, the deviations were toward positive assortment in 11 of these cases. This may suggest the existence of population structure even within a single prefecture.

Ober and others (1997) is the only study to date which has demonstrated negative assortative mating with respect to HLA. Their success may be attributed to a complete knowledge of the birthplaces of the subjects in the study. The Hutterite population studied by Ober and others consists of four "lineages," among which genotype frequencies at HLA loci are known to be different. It is also known that many marriages are endogamous with respect to lineage. Ober and others were able to calculate the expected number of matched haplotypes for each couple based on the genotype frequencies in each lineage, so that the population structure was taken into consideration without reducing the sample size.

The excess of mean number of shared alleles or haplotypes is also consistent with assortative mating preferences. In fact, Yamazaki and others (1976) in a paper reporting male preferences for MHC-dissimilar females in house mice (detected in four of six cases studied), also noted a case of male preference for MHC-similar females (one of the six cases). With respect to humans, Hedrick and Black (1997) observed a deficiency of matings that share no alleles at the HLA-A and -B loci in South Amerindians though the deviations were not significant.

Consanguineous marriages would also increase the number of shared alleles. In the 8JW sample, it is unknown whether consanguineous marriages are present. Imaizumi and others (1975) and Imaizumi (1986) estimated the overall rate of first cousin marriages in Japan at 2.13% and 1.6%, respectively. These studies also showed that the rate of first cousin marriages decreases with the marriage year. Although the exact year of marriage is not known for the couples in the 8JW sample, the rate of first cousin marriages in 8JW is unlikely to be greater than 6% (Imaizumi, 1986). A rough correction for the effect of cousin marriages (at the rate of 6%) can be made by reducing the expected number of matings that share no alleles by 1.5% (= $0.25 \times 6\%$) and redistributing them among the expected numbers of matings that share one or two alleles. The *G*-statistics were not significant on the assumption that they follow the chi-square distribution with 2 degrees of freedom. We suggest, therefore, that our failure to detect disassortative mating is not due to the possible inclusion of consanguineous marriages in 8JW.

It is not known whether our samples included arranged marriages. If there were arranged marriages, this might have limited the exercise of the subjects' mate choice based on HLA similarity. Arranged marriages have been decreasing consistently in Japan. The proportion of arranged marriages is

estimated at 9.9% among the people who were married in and after 1995 while it was 49.8% among the marriages during the period from 1960 to 1964, and 69.0% from 1930 to 1938 (National Institute of Population and Social Security Research, 1998). Blood (1967), based on his data collected in 1950's, suggested that young people in Japan had considerable opportunities to choose their own mates and were rarely forced into unwilling marriages even in the case of arranged marriages.

REFERENCES

Adams, M.S. and Neel, J.V. (1967) Children of incest. Pediatrics, vol. 40, pp. 55-62.

Bittles, A.H. and Neel, J.V. (1994) The costs of human inbreeding and their implications for variations at the DNA level. Nature Genetics, vol. 8, pp. 117-121.

Black, F.L. and Salzano, F.M. (1981) Evidence for heterosis in the HLA system. American Journal of Human Genetics, vol. 33, pp. 894-899.

Blood, R.O., Jr. (1967) Love Match and Arranged Marriage - A Tokyo-Detroit Comparison. The Free Press, New York.

Brown, J.L. and Eklund, A. (1994) Kin recognition and the major histocompatibility complex: an integrative review. The American Naturalist, vol. 143, pp. 435-461.

Buss, D.M. (1989) Sex differences in human mate preferences: Evolutionary hypotheses tested in 37 cultures. Behavioral and Brain Sciences. vol. 12, pp. 1-49.

Carrington, M., Nelson, G.W., Martin, M.P., Kissner, T., Vlahov, D., Goedert, J.J., Kaslow, R., Buchbinder, S., Hoots, K., and O'Brien, S.J. (1999) HLA and HIV-1: Heterozygote advantage and B*35-Cw*04 disadvantage. Science. vol. 283, pp. 1748-1752.

Egid, K. and Brown, J. L. (1989) The major histocompatibility complex and female mating preferences in mice. Animal Behaviour. vol. 38, pp. 548-550.

Fisher, R.A. (1954) Statistical Methods for Research Workers. 12th ed. Oliver and Boyd, Edinburgh.

Fujii, Y., Juji, T. and Kaihara, S. (1983) Family study of HLA-A, B, C, and DR in Japanese. Japanese Journal of Transplantation vol. 18, pp. 189-203. [In Japanese]

Hedrick, P.W. (1994) Evolutionary genetics of the major histocompatibility complex. The American Naturalist. vol. 143, pp. 945-964.

Hedrick, P.W. and Black, F.L. (1997) HLA and mate selection: no evidence in South Amerindians. American Journal of Human Genetics. vol. 61, pp. 505-511.

Imaizumi, Y. (1986) A recent survey of consanguineous marriages in Japan. Clinical Genetics. vol.30, pp. 230-233.

Imaizumi, Y., Shinozaki, N., and Aoki, H. (1975) Inbreeding in Japan: results of a nation-wide study. Japanese Journal of Human Genetics. vol. 20, pp. 91-107.

Jin, K., Speed, T.P. and Thomson, G. (1995) Tests of random mating for a highly polymorphic locus: application to HLA data. Biometrics. vol. 51, pp. 1064-1076.

Ihara et al. 2000. Anthropological Science 108(2), 199-214.

Klein, J. (1986) Natural History of the Major Histocompatibility Complex. Wiley, New York.

National Institute of Population and Social Security Research (1998) Marriage and Fertility in Present-day Japan. [In Japanese]

Ober, C., Weitkamp, L.R., Cox, N., Dytch, H., Kostyu, D. and Elias, S. (1997) HLA and mate choice in humans. American Journal of Human Genetics. vol. 61, pp. 497-504.

Penn, D., and Potts, W. (1998) How do major histocompatibility complex genes influence odor and mating preferences? Advances in Immunology. vol. 69, pp. 411-436.

Penn, D., and Potts, W. (1999) The evolution of mating preferences and major histocompatibility complex genes. The American Naturalist. vol.153, pp. 145-164.

Pollack, M.S., Wysocki, C.J., Beauchamp, G.K., Braun, D. Jr., Callaway, C. and Dupont, B. (1982) Absence of HLA association or linkage for variations in sensitivity to the odor androstenone. Immunogenetics. vol. 15, pp. 579-589.

Potts, W.K., Manning, C.J. and Wakeland, E.K. (1991) Mating patterns in seminatural populations of mice influenced by MHC genotype. Nature. vol. 352, pp. 619-621.

Rosenberg, L.T., Cooperman, D. and Payne, R. (1983) HLA and mate selection. Immunogenetics. vol. 17, pp. 89-93.

Schrage, L. (1979) A more portable Fortran random number generator. ACM Transactions on Mathematical Software. vol. 5, pp. 132-138.

Seemanová, E. (1971) A study of children of incestuous matings. Human Heredity. vol. 21, pp. 108-128.

Sokal, R.R. and Rohlf, F.J. (1995) Biometry. 3rd ed. W. H. Freeman and Company, New York.

Takahashi, K., Tokunaga, K., Uchikawa, C., Juji, T., Akaza, T., Umezawa, K., Endoh, N., Kamata, H., Tsuchida, H., Fujino, K. and Watanabe, I. (1992) Evaluation of an appropriate size of unrelated donor pool for HLA-matched platelet transfusion and bone marrow transplantation. Japanese Journal of Transfusion Medicine. vol. 38, pp. 475-481. [In Japanese]

Thursz, M.R., Thomas, H.C., Greenwood, B.M., and Hill, A.V.S. (1997) Heterozygote advantage for HLA class-II type in hepatitis B virus infection. Nature Genetics. vol. 17, pp. 11-12.

Wedekind, C., Seebeck, T., Bettens, F. and Paepke, A.J. (1995) MHC-dependent mate preferences in humans. Proceedings of the Royal Society of London. Series B. vol. 260, pp. 245-249.

Wedekind, C. and Füri, S. (1997) Body odor preferences in men and women: do they aim for specific MHC combinations or simply heterozygosity? Proceedings of the Royal Society of London. Series B. vol. 264, pp. 1471-1479.

Yamazaki, K., Beauchamp, G.K., Kupniewski, D., Bard, J., Thomas, L., and Boyse, E.A. (1988) Familial imprinting determines H-2 selective mating preferences. Science. vol. 240, pp. 1331-1332.

Yamazaki, K., Boyse, E.A., Mike, V., Thaler, H.T., Mathieson, B.J., Abbott, J., Boyse, J., Zayas, Z.A. and Thomas, L. (1976) Control of mating preferences in mice by genes in the major histocompatibility complex. The Journal of Experimental Medicine. vol. 144, pp. 1324-1335.

Ihara et al. 2000. Anthropological Science 108(2), 199-214.

Yamazaki, K., Yamaguchi, M., Baranoski, L., Bard, J., Boyse, E.A. and Thomas, L. (1979) Recognition among mice. Evidence from the use of a Y-maze differentially scented by congenic mice of different major histocompatibility types. The Journal of Experimental Medicine. vol. 150, pp. 755-760.