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HLA and Mate Selection: No Evidence in South Amerindians

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Summary

There have been reports of nonrandom mating (negative-assortative mating) or preference for individuals of different major histocompatibility complex (MHC) genotypes in both mice and humans. We have examined the association of HLA-A and HLA-B genotypes, both for each locus by itself and for two-locus genotypes, in mates of 194 couples from 11 South Amerindian tribes. The proportion of couples sampled averaged >50% of the total matings with progeny for 10 of the tribes. In nearly all cases, HLA-sharing proportions were very close to those expected from random mating, suggesting strong negative-assortative mating for MHC is not present in these South Amerindians.

Introduction

The factors thought to influence genetic variation at the major histocompatibility complex (MHC) (HLA in humans) encompass nearly every facet of population genetics (e.g., see Hedrick 1994; Parham and Ohta 1996). In particular, balancing selection appears to be important in maintaining the large amount of variation observed at many MHC genes, and the selective effects of different pathogens or parasites appear to be the driving force in the maintenance of MHC variation (e.g., see Parham and Ohta 1996; Hedrick and Kim, in press). However, selection resulting from both maternal/fetal interaction and negative-assortative mating may have significant effects on maintaining variation at MHC loci in some species (for reviews, see Alberts and Ober 1993; Potts and Wakeland 1993; Brown and Eklund 1994). Although there is a great deal known about the molecular genetics of MHC, particularly in humans and mice, there is much still unknown about the type and extent of balancing selection, leading to strongly differing opinions about the most significant selective factors influencing genetic variation at MHC genes.

Laboratory studies have demonstrated mating preferences with regard to MHC types in mice (known as "H-2" types) (for a review, see Brown and Eklund 1994). There are studies showing both that males mate preferentially with females of a different H-2 type (e.g., see Yamazaki et al. 1976; however, also see Eklund et al. 1991) and that females select males of a different H-2 type (Egid and Brown 1989). On the basis of cross-fostering experiments, it appears that male preference may be the result of familial imprinting (Yamazaki et al. 1988). In a seminatural mouse population, Potts et al. (1991) found a large deficiency of H-2 homozygotes and concluded, by eliminating other factors, that this effect was due to female selection of mates, different at H-2, from themselves. Hedrick (1992) showed that, if there was strong negative-assortative mating by females, a deficiency in homozygotes that is as high as that observed by Potts et al. (1991) could be generated.

There is no published evidence for nonrandom mating with respect to HLA, although there are reports consistent with random mating (e.g., see Pollack et al. 1982; Rosenberg et al. 1983; Jin et al. 1995). However, the demonstration of negative-assortative mating in humans is difficult, because the high polymorphism at many HLA loci makes nearly all mating types rare and makes those that share two alleles infrequent. Only by combining specific mating types, as, for example, in a test for the number of shared alleles between two mates, can negative-assortative mating be examined statistically in most populations (Jin et al. 1995).

Recently, Wedekind et al. (1995) examined preferences of female university students for T-shirts worn by male university students who had either high or low HLA sharing with the females. The results suggested that the females preferred HLA-different males more than HLA-similar males, although in one comparison women on birth control pills had a preference for HLA-similar males (see Discussion). These conclusions have resulted in extensive speculation about the use of perfumes, the divorce rate, and other issues (Vollrath and Milinski 1995; Wedekind et al. 1995), even though various aspects of the study appear to be somewhat questionable (Hedrick and Loeschcke 1996).

HLA variation has been extensively examined in South Amerindians, both by tissue typing (Bhatia et al. 1995, and references therein) and, more recently, by determination of DNA sequence variation (Parham and Ohta 1996, and references therein). In these groups, there is evidence supporting substantial selection fa-

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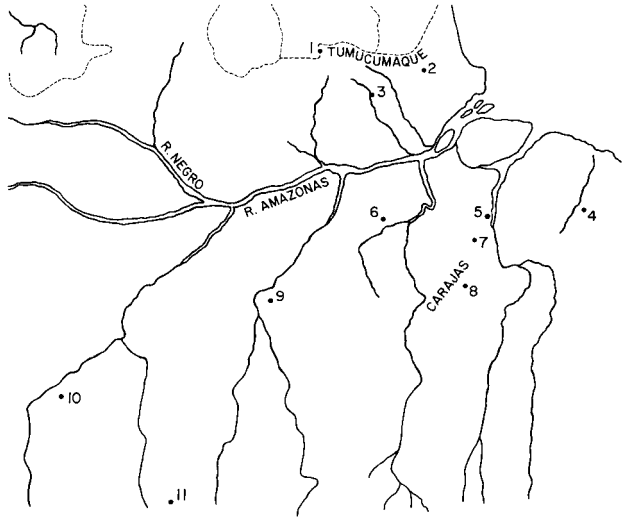

Figure 1 Lower Amazon basin, showing locations of 11 tribal groups included in this study. The numbers denote the tribes, given in the tables.

voring heterozygotes (or some other type of balancing selection): deviations from Hardy-Weinberg proportions (Black and Salzano 1981), deviations from Mendelian expectations in families (Black and Salzano 1981), and more even distributions of allelic frequencies than would be expected under neutrality (Hedrick 1983). Below we examine the level of HLA sharing in 194 couples, all of whom had offspring who were consistent with the parental genotypes, from 11 remote tribes of South Amerindians, each with $\geqslant$10 couples, to determine whether there is a difference from the mating frequencies expected under random mating within the tribe.

Subjects, Material, and Methods

Samples and Extent of Genetic Variation

The locations of the 11 tribes sampled are given in figure 1. These tribes are physically quite separated from each other and speak different languages, often from different language groups (e.g., see Bhatia et al. 1995), and there is no known history of exchange between them. Our samples included only couples that had offspring with genotypes consistent with the parental genotypes. In other words, these couples were ones that had reproduced successfully and therefore constitute a biologically meaningful investment compared with either couples determined only by odor preference or couples with no offspring. The couples sampled constitute a high proportion of the couples with children in 10 of the 11 tribes; the exception is the Munurucu (this large group had $>$300 couples at the time of sampling; however, our sample of 15 couples was a majority of the couples with children from the sampled village). The estimated proportion of couples sampled for the other 10 tribes ranged from .30 for the Urubu-Kaabor to .81 for the Arara, with a mean of .52.

These individuals were typed for HLA-A and HLA-B by use of traditional serotyping protocols (e.g., see Black et al. 1980; Black and Salzano 1981). For HLA-A, four different alleles were observed—02, 24, 31, and 68—and, for HLA-B, five different alleles were observed—05, 15, 35, 39, and 40. Nearly all populations had all alleles—or all alleles except one—at both loci. There is no evidence of serological blank alleles in these samples, for either HLA-A or HLA-B, or in the larger population samples from these populations, suggesting that, if blank alleles were present, they were at quite low frequency. The average observed and expected heterozygosities for the mating pairs over all populations were .764 and .654, respectively, for HLA-A and .732 and .692, respectively, for HLA-B; in other words, there was a substantial degree of polymorphism in the populations, but it was much lower than the serotypic variation observed in European or African populations, which may have 15–18 alleles for HLA-A and 25–30 alleles for HLA-B and heterozygosities $>$.9 for both loci (e.g., see Hedrick and Thomson 1983). The observed and expected heterozygosities are statistically significantly different for HLA-A and nearly so for HLA-B, so that observed genotypic frequencies are used in the calculations below (the complete list of one- and two-locus genotypic frequencies for females and males for the different tribes is available from P.W.H., on request).

On the basis of the studies summarized by Parham and Ohta (1996), it appears that three of the serotypic alleles at HLA-A—A*2402, A*31012, and A*68012 (here the first two digits indicate the serotype used above, and the remaining digits indicate a specific nucleotide sequence; serotype 68 previously had been designated as "28")—are probably homogeneous in these tribes, as they are in other South Amerindian tribes, for their nucleotide sequence. The other HLA-A allele, 02, shows heterogeneity in South Amerindians (Parham and Ohta 1996), so that we do not know, without nucleotide information, which 02 sequence(s) is in our samples. The picture for HLA-B is quite different, since every serotype found in the 11 tribes shows heterogeneity in South Amerindians (Parham and Ohta 1996). Parham and Ohta (1996) hypothesize that there was only one founding allele for serotypes 15, 35, and 39—namely, B*1501, B*3501, and B*39011—whereas both 40 and 51 are hypothesized to have two founding alleles (previously, serotypes 15, 40, and 51 had been designated, respectively, as "62," "60," and "5"). As a result, in this study the mating types for HLA-A are less likely to have undetected variation than are those for HLA-B (see Discussion).

Sharing of Alleles in Mating Pairs

The observed sharing of HLA alleles in these pairs, for a given locus, was determined in the following man-

Table 1

Observed and Expected Numbers of Matings That Share or Do Not Share Alleles at HLA-A, in 11 Tribes

Tribe	No. of Matings	Observed/Expected No. of Matings Sharing: No Alleles	One Allele	Two Alleles
1. Tiriyo	19	5/6.63	12/10.47	2/1.89
2. Waiapi	25	3/4.60	17/15.76	5/4.64
3. Apalai	20	0/1.40	15/13.80	5/4.80
4. Urubu-Kaapor	27	6/8.22	14/14.48	7/4.30
5. Asurini Trocara	19	6/3.63	9/11.37	4/4.00
6. Arara	10	2/1.00	6/6.40	2/2.60
7. Paranana Velho	22	0/2.50	19/14.36	3/5.14
8. Xikrin	11	1/1.27	9/7.27	1/2.45
9. Mundurucu	15	3/2.40	12/9.47	0/3.13
10. Karitiana	13	1/1.00	7/8.31	5/3.69
11. Cinta Larga	13	2/2.92	8/7.85	3/2.23
Total[a]	194	29/35.57	128/119.54	37/38.87

[a] $\chi^2 = 1.90$ (df = 2, not significant).

ner. As examples of mating pairs from HLA-A, where the first individual is the female, the sharing is zero from mating 02 02 × 24 68, one from 02 24 × 02 68 or 02 02 × 02 68, and two from 02 24 × 02 24. The expected sharing of alleles in mating pairs was calculated separately for each population, by use of the observed female and male genotypic frequencies in the mating pairs. This removes any bias that may occur if Hardy-Weinberg genotypic frequencies are used, and it allows for differences, in genotypic frequencies, between females and males. Therefore, the expected frequency of a given mating was calculated as $\Sigma P_{ij.f} P_{ij.m}$, where $P_{ij.f}$ and $P_{ij.m}$ are the observed frequencies of the genotype with alleles *i* and *j* in females and males, respectively, in the matings pairs of a given tribe. Each mating pair is then classified as sharing no, one, or two alleles, as shown above, and the mating pairs were summed over each of these mating categories. The same approach was extended to two loci where the genotypic frequencies were the observed two-locus female and male genotypic frequencies. The mating pairs were then categorized as sharing zero to four alleles and were summed over the appropriate mating category. We did not examine sharing of two-locus haplotypes, because the two-locus genotypic frequencies give the sum of the two haplotypes in an individual and because we are unable to determine or estimate accurately the haplotypes in families with only one (or a few) offspring and small numbers in the tribal samples.

In addition, the sharing between mates of specific alleles at a locus was also examined; for example, for allele 02 at HLA-A, a female and a male may share allele 02 (e.g., 02 24 × 02 31), the female may have 02 and the male may not (e.g., 02 24 × 24 24), the female may not have 02 and the male may not (e.g., 24 31 × 02 02), or neither may have it. In general, we can indicate these four classes as *i,i; i,$\bar{i}$; $\bar{i}$,i;* and $\bar{i},\bar{i}$, where the first and second letters refer to the female and the male, respectively, and the absence or presence of an overbar indicates that the individual does or does not have allele *i*. The expected values were calculated by use of the observed frequencies of a given allele in females and males in each population, and the expected and observed numbers were summed over tribes. Only tribes in which an allele was present in both sexes were used. In this case, because allelic frequencies must sum to unity within a tribe, three of the allelic comparisons for HLA-A are independent and four allelic comparisons for HLA-B are independent.

Results

Tables 1 and 2 give the observed and expected number of mating pairs that share no, one, or two alleles at HLA-A and HLA-B, for the 11 tribes. Overall, there is no evidence of an excess of observed matings that share no alleles, and, in fact, for both loci there is a slight deficiency of matings that share no alleles (and a slight excess of matings that share two alleles for HLA-B), the opposite of what negative-assortative mating for HLA would predict. For HLA-A and HLA-B, the observed numbers of matings that shared no alleles were 29 and 44, respectively, whereas the expected numbers for both loci were higher—35.57 and 50.55, respectively. There was no overall statistical significance between the observed and expected numbers for either locus, with a value of $\chi^2 = 1.90$ (2 df) and $\chi^2 = 4.14$ (2 df) for HLA-

Table 2

Observed and Expected Numbers of Matings That Share or Do Not Share Alleles at HLA-B, in 11 Tribes

Tribe	No. of Matings	Observed/Expected No. of Matings Sharing: No Alleles	One Allele	Two Alleles
1. Tiriyo	19	3/5.84	14/11.00	2/2.16
2. Waiapi	25	7/6.72	15/14.80	3/3.48
3. Apalai	20	8/7.50	11/10.95	1/1.55
4. Urubu-Kaapor	27	3/10.48	14/13.41	10/3.11
5. Asurini Trocara	20	7/4.35	9/12.00	4/3.65
6. Arara	10	1/2.60	8/6.30	1/1.10
7. Paranana Velho	22	1/1.86	17/14.45	4/5.68
8. Xikrin	22	3/2.64	5/6.55	3/1.82
9. Mundurucu	15	9/5.07	4/8.07	2/1.87
10. Karitiana	13	1/.85	8/9.00	4/3.15
11. Cinta Larga	13	2/3.15	7/7.38	4/2.46
Total[a]	194	44/50.55	112/113.54	38/29.91

[a] $\chi^2 = 4.14$ (df = 2, not significant).

Table 3

Observed and Expected Numbers of Matings That Share or Do Not Share Alleles at HLA-A and HLA-B, in 11 Tribes

Tribe	No. of Matings	Observed/Expected No. of Matings Sharing				
		No Alleles	One Allele	Two Alleles	Three Alleles	Four Alleles
1. Tiriyo	19	2/2.53	3/6.26	11/7.58	3/2.37	0/.26
2. Waiapi	25	1/1.88	5/6.08	16/11.12	1/4.56	2/1.36
3. Apalai	20	0/.45	7/5.85	9/9.43	4/4.00	0/.25
4. Urubu-Kaapor	27	0/4.00	8/8.52	9/9.96	6/3.85	4/.67
5. Asurini Trocara	19	4/1.89	5/3.47	4/7.53	3/4.89	3/1.21
6. Arara	10	0/.30	3/2.60	4/4.30	3/2.60	0/.20
7. Paranana Velho	22	0/1.27	2/3.32	15/7.45	3/6.41	2/3.55
8. Xikrin	11	1/.36	2/2.55	5/4.91	3/2.73	0/.45
9. Mundurucu	15	3/1.19	6/4.31	4/5.81	2/3.06	0/.62
10. Karitiana	13	1/.77	0/.38	7/7.85	1/1.08	4/2.92
11. Cinta Larga	13	2/1.38	0/3.00	5/4.92	5/2.92	1/.77
Total[a]	194	14/16.02	41/46.36	89/80.86	34/38.43	16/12.26

[a] $\chi^2 = 3.34$ (df = 4, not significant).

A and HLA-B, respectively. For HLA-A, observed and expected values were very close for all tribes. For HLA-B, the biggest difference (deficiency) was in the Urubu-Kaabor, in which only three matings shared no alleles, whereas 10.48 were expected, and in which 10 matings shared two alleles, whereas only 3.11 were expected. The Mundurucu showed a somewhat smaller difference (excess) of matings in which no alleles were shared; 9 were observed, whereas 5.07 were expected. For the other nine tribes, the observed and expected values were similar for all mating categories.

To allow determination of whether an effect not seen at a single locus may become apparent when both loci are simultaneously examined, table 3 gives the observed and expected matings for two loci. Again, there is a slight deficiency of matings that share no alleles—14 observed versus 16.02 expected—and a slight excess of matings that share all four alleles—16 observed versus 12.26 expected. In other words, the trend again is the opposite of that expected for negative-assortative mating. The differences between observed and expected are again not statistically significant ($\chi^2 = 3.34$, 4 df).

To allow determination of whether there is nonrandom mating with respect to a given allele, table 4 gives the observed and expected matings for each allele separately. In this case, the observed and expected numbers are again very close to each other, and none of the χ^2 values are significant (the sample sizes vary by allele because some alleles were missing from some tribes). The largest χ^2 value is for allele 15 at HLA-B, where there is an excess of observed over expected, both for the category in which the mates share allele 15 (40 observed vs. 31.95 expected) and for the category in which they both do not have allele 15 (78 observed vs. 69.95 expected).

A large proportion of the excess (4.00 of 8.05) for both categories is from the Urubu-Kaapor, in which 7 matings that shared allele 15 were observed, whereas only 3.0 were expected, and in which 16 matings in which neither partner had allele 15 were observed, whereas only 12.0 were expected. There is an excess of homozygotes over heterozygotes in the Urubu-Kaabor (22 homozygotes observed vs. 15.42 expected, the opposite of what is observed in the other tribes [F. L. Black, unpublished data]), suggesting that two (or more) groups that differed in allele frequencies may have been

Table 4

Observed and Expected Numbers of Matings Summed over 11 Tribes, Where First Allele Is That of the Female and Second Is That of the Male

Locus and Allele	Observed/Expected No. of Matings				χ^2
	i,i	$i,\bar{i}$	$\bar{i},i$	$\bar{i},\bar{i}$	
HLA-A:					
02	72/69.07	42/44.93	42/44.93	38/35.07	.74
24	53/51.43	36/37.57	40/41.57	43/41.43	.24
31	45/47.44	42/38.56	39/37.56	58/60.44	.54
68	14/13.69	17/17.31	23/23.31	86/85.69	.02
HLA-B:					
15	40/31.95	22/30.05	32/40.05	78/69.95	6.74
35	75/73.92	42/43.08	41/42.08	36/34.92	.11
39	22/24.93	37/34.07	26/23.07	72/74.93	1.07
40	26/24.65	33/34.35	34/35.35	78/76.65	.18
51	11/10.54	24/24.46	24/24.46	57/56.45	.04

combined (the Wahlund effect; e.g., see Hedrick 1985). If matings also took place separately in two (or more) separate groups, then the excess of like × like matings could be explained. However, although the Urubu-Kaabor are composed of several components, they do not appear to be strongly endogamous, and there is considerable exchange among villages; in other words, the excess of homozygotes and like × like matings is not explainable on the basis of the known anthropological information and may be just a chance result.

Discussion

Mating frequencies for HLA-A and HLA-B in a sample that constitutes a large proportion of the total number of matings with progeny from South Amerindians from 11 different tribes are consistent with random-mating expectations. In fact, there is an observed deficiency of matings that do not share alleles for HLA-A and HLA-B, the opposite of what is expected for negative-assortative mating.

Estimate of Selection and Statistical Power

Even though we have examined a large proportion of all the couples with offspring, within all tribes (except for the Mundurucu), and even though the observed matings differed from random-mating expectations, in the direction opposite of negative-assortative mating, we should determine what level of negative-assortative mating we could detect in this sample. As background, let us mention the main cases in which preference has been shown. First, in mice, the level of male-mate selection between different H-2 types, found by Yamazaki et al. (1976), was .57 (a value of 0 indicates no differential selection, and the maximum selection is 1.0; Hedrick 1994); the level of female-mate selection between different H-2 types, found by Egid and Brown (1989), was .66; and the level of selection necessary to explain the deficiency of heterozygotes in the study by Potts et al. (1991) was .69 (Hedrick 1992).

Second, Wedekind et al. (1995) examined the preference of female university students for T-shirts worn by male students who were either dissimilar or similar to the females at HLA-A, HLA-B, and HLA-DR (each of these preference values is the mean of three T-shirt scores of different men). The mean pleasantness scores *per female* were 5.61 and 4.66 for dissimilar and similar males, respectively, when the women were not on birth-control pills ($N = 31$) and were 5.03 and 4.60, respectively, when the women were on birth-control pills ($N = 18$). The mean pleasantness scores *per male* were 5.71 and 4.64 for dissimilar and similar males, respectively, when the women were not on birth-control pills ($N = 38$) and were 4.58 and 5.76, respectively, when the women were on birth-control pills ($N = 23$). When these scores are standardized for the highest value in each comparison, the estimated proportional differences in these four comparisons are .17, .08, .19, and .20, respectively (in this last comparison, the similar HLA type is favored), all of which, except for the second one, Wedekind et al. (1995) found to be statistically significant.

Hedrick and Loeschcke (1996), in evaluating the study by Wedekind et al. (1995), made several points that are relevant here. Wedekind et al. (1995) had no measure of experimental error that could have been obtained by presenting the same T-shirt to the same woman more than once. The reversal in preference among women on birth-control pills, depending on whether the per-female or per-male mean is used, is particularly worrisome and was not explained. Finally, the preference examined is for a T-shirt odor, a preference that is probably based on criteria quite different than those used by women to choose a mate for reproduction.

A simple estimate of the statistical power to detect mating based on HLA can be obtained by weighting the values expected from random mating (see tables 1 and 2), by 1, $1 - s/2$, and $1 - s$, for no alleles shared, one allele shared, and two alleles shared, respectively, and then standardizing them to obtain the same total sample size. For both HLA-A and HLA-B, an s value of .45 gives a χ^2 value that is significant at the .05 level. In other words, we do not have enough statistical power in these data to detect a selective difference of $<.45$, given the negative-assortative mating model above. This should have allowed us to detect the extent of selection observed in the mouse experiments discussed above but would not enable us to detect the level of difference observed by Wedekind et al. (1995), even though our sample size is larger.

We should note that, because of the low polymorphism found in the tribes that we examined, we have a similar number of matings that share either no or two alleles. In more polymorphic populations, such as most African, Asian, and European populations, a very high proportion of matings would share no alleles, and nearly all the rest would share one allele. Because of the very low numbers of matings that would share two alleles, there is less statistical power to detect matings related to HLA differences in these populations, given the same sample size, than in our less polymorphic samples.

Marriage Preferences

There is substantial variation, over these tribes, in marriage preferences; for example, there does appear to be a socially dictated preference for marriage between cross-cousins—that is, matings with either mother's brother's child or father's sister's child, which are two of the four types of first-cousin matings—in some Amerindians. In an ethnographic sample, Murdoch (1957) lists 77 Central and South American tribes and states that 12 (16%) preferred cross-cousin marriage. Two of these tribes, the Apalai and the Mundurucu, are included

in our sample. The known genealogies for these tribes are not deep, but they reveal no evidence of this preference. Either the preference is not rigidly enforced or, in these populations, where each member is related to every other member through several lines of descent, it can be interpreted to pose little restriction. This, coupled with the small proportion of tribes that appear to have such a preference, makes it unlikely that a substantial effect due to personal preference for HLA-dissimilar types would be masked by such cultural preferences, which would increase sharing of HLA in mates.

In addition, the determination of mates varies greatly over these tribal groups; for example, Kayapo women appear to be potent and free to choose any male partner and to marry him after they become pregnant, whereas the Parakana woman appears to be passed in marriage according to rules that favor dominant men. Other tribes appear to fall between these extremes. There is no evidence of the arrangement of marriage, as in some other cultures, except for some of the matings between relatives, as discussed above, and these do not appear to be common in our samples.

In these tribes there is little evidence of matings between very close relatives, such as brother-sister, father-daughter, or uncle-niece matings. Matings between close relatives would presumably result in higher than average sharing of HLA types, and their avoidance would somewhat bias mating frequencies, toward lower than expected HLA sharing, or the appearance of negative-assortative mating. This effect, in theory, may increase the frequencies of heterozygotes in very small populations (for discussion, see Markow et al. 1993) and could have a similar effect on mating frequencies. However, we actually observed higher than expected—not lower than expected—HLA sharing, suggesting that, if such an influence is important in these tribes, other factors must have masked it.

Other Considerations

If there is undetected heterogeneity within serotypes, some matings thought to share two alleles would share only one or no alleles, and some matings thought to share one allele would share none. As discussed above, within and between South Amerindians tribes, HLA-A has very little heterogeneity within serotype, whereas HLA-B appears to have substantially more. Because both loci are similarly consistent with random-mating proportions in this study, we feel that it is unlikely that heterogeneity within serotypic class has a substantial impact on mate selection.

In the laboratory-mouse studies (e.g., see Yamazaki et al. 1976; Egid and Brown 1989), individuals were homozygous for all loci and were genetically identical for all loci—except for the H-2 region, in which the strains differed. In the study by Potts et al. (1991), again the genetic background was identical, but mice were variable for different H-2 haplotypes. It is possible that the homogeneity of the genetic background in these studies may accentuate any differences in the MHC region (see below). It is also possible that the MHC region that differs between haplotypes in these artificially constructed mouse lines is larger than that in our study of a natural population. In these mouse studies, as well as in the human study by Wedekind et al. (1995), MHC differences included both class I and II loci, whereas in the present study we have data only on class I loci. It was not known or reported in these other studies whether the two categories of loci contribute differentially to the significant mating effects, but, if the effect is primarily due to class II loci, then we would have have detected only an effect resulting from linkage disequilibrium between class I and II loci.

In our examination of HLA-A and HLA-B simultaneously, we considered two-locus genotypes, not two-locus haplotypes. As stated above, we did not have either enough progeny from given matings or enough population data to adequately estimate haplotype frequencies. It is not obvious how mate selection could be based only on haplotypes and be independent of genotypes. The genotype (or phenotype) is the only possible unit involved in nonrandom mating in a natural population, and whether it contains particular haplotypes depends on the extent of linkage disequilibrium between loci. In any case, the level of haplotype sharing should be reflected in the level of sharing at the genotypic level. Furthermore, the examination of sharing of individual two-locus haplotypes results, even in our rather unpolymorphic sample, in 20 potential two-locus haplotypes, which, in turn, would make many classes too small for satisfactory statistical analysis.

It is possible that, in controlled laboratory studies with congenic lines (e.g., see Yamazaki et al. 1976, 1988; Egid and Brown 1989), in simplified environments such as the seminatural enclosures of Potts et al. (1991), or in human odor-preference trials in which other odors are eliminated or reduced (Wedekind et al. 1995), various genetic, environmental, or cultural factors would be eliminated and that MHC would become a stronger cue for mate choice. On the other hand, it is often thought that selection in laboratory experiments is reduced relative to that present in nature. Although there may be different and perhaps substantial selective pressures in a laboratory environment, there may in fact be relaxed selection on other factors necessary for survival and reproduction in natural populations. In the populations examined here, the individuals chose mates in the way traditional to their tribes, and this presumably includes the types of selection that have always existed in these groups. In other words, the mate selection that we have observed may be stronger and/or more a reflection of natural selective forces than preferences in a less natural situation.

Obviously, if the odor signal for mating observed in controlled situations is overwhelmed by other genetic, environmental, or cultural factors in a more natural situation, its importance in influencing evolutionary change may be of minor significance. In light of this, it seems important to determine the extent of mate choice for MHC in reproductive pairs in natural populations of mice or in couples that produced offspring in the German Swiss population examined by Wedekind et al. (1995). In a recent study in pheasants, von Schantz et al. (1996) observed that male survival and spur length were associated with MHC genotypes; however, they presented no information on MHC-genotype similarity between these males and their female mates.

There does appear to be strong selection related to HLA—but unrelated to mate selection—acting in these tribes (e.g., see Black and Salzano 1981; Hedrick 1983, 1997). In other words, in the same groups in which we find no evidence of mate choice based on HLA, there are large selective effects influencing allelic frequencies, genotypic frequencies, and progeny frequencies, from known parents.

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