

Title: A population-level test of human negative assortative mating along HLA class I and class II loci.

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Abstract

Many studies have recently explored the role of HLA (human leukocyte antigen) dissimilarity in human mate choice. These studies were prompted by earlier research that showed that mice were more likely to mate with individuals that were dissimilar at MHC (major histocompatibility complex) loci than random chance would predict. Researchers have employed two main methodologies--odor preference and mateship studies—to discern whether humans disassortatively mate along HLA loci and have found mixed results. This paper uses these datasets to determine if levels of heterozygosity among these populations deviate significantly from Hardy-Weinberg expectations due to the proposed negative assortative mating patterns. No significant deviations were found in the predicted direction.

INTRODUCTION

The HLA (human leukocyte antigen) complex, referred to as MHC (major histocompatibility complex) in other animals, is a highly polymorphic gene complex that is crucial to the proper functioning of the immune system (Klein 1986). This gene complex plays an important role in initiating most immune system responses by discriminating between self and non-self, thereby enabling pathogen detection (Thornhill and others 2003). Because of this function, the high levels of polymorphism found among these loci are thought to be maintained by host-pathogen co-evolution (Hughes and Hughes 1995). Quickly evolving pathogens are able to adapt to common or long-lasting alleles, providing a selection advantage to novel alleles or allelic combinations.

Many studies have shown that mice preferentially mate with individuals that are dissimilar to themselves at MHC loci (Penn 2002; Potts and others 1991; Yamazaki and others 1976). The mice specifically prefer the scent of other mice that are dissimilar to the scent of individuals that were in their natal group, which would usually consist of kin (Yamazaki and others 1988).

There are four proposed adaptive explanations for the preference for dissimilar individuals (Penn and Potts 1999; Thornhill and others 2003). Disassortative mating results in higher levels of heterozygosity among offspring. The heterozygosity hypothesis argues that the higher levels of offspring heterozygosity caused by disassortative mating afford the offspring immunocompetence against a greater number of pathogen types, given that these alleles are codominant (Wedekind and others 1995). Consistent with this hypothesis, mice that are MHC heterozygous have been found to be more able to resist multiple strains of pathogens than their homozygous counterparts (Penn and others 2002). Additionally, Black and Hedrick (Black and Hedrick 1997) found that there were fewer homozygous children of South American Indians than would be predicted, given the genotypes of their parents and normal Mendelian segregation, implying some advantage to heterozygotes early in life. The diverse-genes hypothesis posits that the preference for dissimilar individuals functions as a method of obtaining alleles different from the parent's alleles. This continuous variation may provide a "moving target" to which pathogens are less adapted (Ihara and others 2000). Similarly, the rare-allele hypothesis implies an ostensible preference for dissimilar individuals that functions to create offspring that will be more resistant to pathogens. Since the majority of individuals will have common alleles, a preference for rare alleles in mates would result in greater dissimilarity between mated pairs. These rare alleles may confer the ability to recognize rapidly evolving pathogens that may go undetected by immune systems containing more common alleles. Thornhill et al. (2003), however, failed to find any preference for the scent of rare alleles in humans. Finally, due to the polymorphic nature of MHC loci, individuals that are similar with respect to MHC alleles are likely to be related, and a preference for dissimilar individuals could aid in avoiding inbreeding, which is often deleterious.

In humans, researchers have employed two main methodologies for detecting a preference for mates that are dissimilar with respect to HLA alleles. In one method, researchers measure odor preferences, based on the belief that HLA dissimilarity is detected through olfaction (Jacob and others 2002). Wedekind et al. (1995) had men wear t-shirts for 2 nights and asked women to rate the attractiveness of their scents. Both the t-shirt wearers and the raters were typed for HLA loci. They found that women that were not using oral contraceptives preferred the scents of men who had HLA genotypes dissimilar to their own. A second study replicated this finding and found that men had a similar preference for the scent of dissimilar women as well (Wedekind and Furi 1997). Thornhill et al. (2003), using similar methods, found a preference for the scent of dissimilar individuals in men, but no such preference in women. Jacob et al. (2002)

found an effect opposite that predicted by negative assortative mating. Women were asked to rank the odor of t-shirts worn by six men. The wearers of the t-shirts with highest ranked odors shared a significantly greater number of HLA alleles with the ranker than did those of the least preferred. This was only true, however, along paternally inherited alleles. Because this study dealt with a lower range of HLA differences, it was argued that the findings support the hypothesis that an intermediate level of dissimilarity is optimal.

Researchers have also explored the levels of dissimilarity between married couples in order to detect a preference for mates that are dissimilar along HLA loci. A study of Hutterite couples (Ober and others 1997) revealed that married couples were more HLA-dissimilar than would be expected by chance, even after accounting for the population's small size and unique structure. Other studies, however, among South American Indians (Hedrick and Black 1997) and Japanese couples (Ihara and others 2000), found no such evidence of negative assortative mating.

Although previous studies have focused on more direct methods of measuring preference for HLA-dissimilar individuals, this paper will examine population effects of negative assortative mating, in particular the expected augmentation of rates of heterozygosity relative to that expected under Hardy-Weinberg equilibrium. As individuals preferentially choose dissimilar mates, parents are less frequently able to pass identical alleles on to their offspring, resulting in decreased levels of homozygosity and increased levels of heterozygosity. Chen et al. (1999) tested for Hardy-Weinberg equilibrium for four class II HLA loci in 26 populations and found significant deviations from expected genotypic frequencies in only 19 of 99 goodness-of-fit tests. Rivas et al. (1997) found only 12 out of 102 populations deviated from Hardy-Weinberg equilibrium for the HLA-DQA1 locus, 8 with decreased levels of heterozygosity and 4 with increased levels. Similarly, Soares-Vieira et al. (1999) found no significant deviation for the HLA-DQA1 locus in a Brazilian population. These studies, however, did not specifically test for an increased number of heterozygotes, but only deviation from Hardy-Weinberg frequencies for all possible genotypes, and, except for Rivas et al. (1997), did not report whether the deviations resulted in greater frequencies of heterozygotes. A deviation from Hardy-Weinberg expectations may result from numerous deviations of specific genotypic frequencies, while levels of total heterozygotes and homozygotes may conform to expectations. Additionally, these studies did not test for the class I loci for which researchers have found evidence suggestive of negative assortative mating.

Because one of the proposed benefits of negative assortative mating along HLA loci predicts, and some evidence has revealed, a possible selective advantage for heterozygous individuals, higher frequencies of heterozygotes may not be evidence of such mating patterns, but simply random mating and subsequent selection. Failing to find such an increase, however, with a test of significant power would be strong evidence against it.

METHODS

Datasets

The datasets compiled in the studies listed above were utilized to test for deviations from Hardy-Weinberg expectations—specifically, those of Ihara et al. (2000) (Japanese population), Thornhill et al. (2003) (North American Caucasian and Hispanic population), Jacob et al. (2002) (German I population), and Wedekind and Furi (1997) (Germany II population) were utilized. The two German datasets were not combined due to differing allelic frequencies. If the two datasets constitute subpopulations, pooling them would result in reduced levels of

heterozygosity (Wahlund 1928). In the North American and for HLA-DR in the Germany II datasets, DNA was typed following polymerase chain reaction (PCR) for the HLA loci. For the Japanese and Germany I datasets, and for HLA-A and HLA-B in the Germany II dataset, HLA genotypes were determined by serology (Takahashi and others 1992). For detailed descriptions of these methodologies, refer to the individual references.

Statistical Methods

Allelic frequencies were determined in each sample by using the gene count method (Li 1976). The expected frequency of heterozygotes was calculated as:

$$H = 1 - (p_1^2 + p_2^2 + \dots + p_n^2)$$

in which p represents the frequency of a particular allele.

The observed frequency of heterozygotes was tested against the prediction using a X^2 goodness-of-fit test. Because theory provides a predicted direction, the goodness-of-fit is tested at the 0.10 level.

RESULTS

Descriptive statistics are provided in Table 1. The four groups vary considerably in their sample sizes and number of alleles. It appears that the serology method of HLA typing, used in the Japan, Germany I, and two of the loci of Germany II datasets, may underestimate the number of alleles, although the expected and observed frequencies of heterozygotes were comparable to the other data sets. Allele frequencies also vary widely, as shown in Figure 1. For this reason, the populations were analyzed separately.

No populations showed levels of heterozygosity that were significantly higher than those expected under Hardy-Weinberg equilibrium for any of the three loci (Table 2). The Germany II sample actually had significantly decreased frequencies of heterozygotes for both the HLA-A and HLA-B loci. Out of a total of 15 tests, only 8 showed levels of heterozygosity higher than expected, none of which were significant.

It is important to note that although frequencies of heterozygotes did not deviate significantly from Hardy-Weinberg expectations in the analyses presented in this paper, this does not necessarily mean that the populations are in Hardy-Weinberg equilibrium. Specific genotypic frequencies may significantly vary. Frequencies of heterozygous genotypes with excesses may be offset by others with deficiencies. Only the combined effects on total levels of heterozygosity are presented in this paper.

DISCUSSION

These populations show no significant increase in heterozygosity among HLA loci as would be expected with substantial negative assortative mating. Additionally, selection against homozygotes, like that found among the South Amerindians (Hedrick and Black 1997), does not seem to be appreciable within these populations. Although heterozygous individuals may enjoy greater immunocompetence, this advantage may be offset by the enhanced diet, sanitation, and medical access of Western populations.

Some effects may act to dilute the population-level effects of negative assortative mating. Population substructure can lead to reduced levels of heterozygosity. Even if there is random mating within subpopulations, differences in allele frequencies between subpopulations result in a reduction in levels of heterozygosity from those expected from pooled allelic frequencies (Wahlund 1928). Ihara et al. (2000) found significant differences in HLA-B allele frequencies among the 6 prefectures from which their data were sampled. The extent of population substructure in the other four samples is unknown. Wedekind et al. (1995) found that women using oral contraceptives exhibited no preference for HLA dissimilar individuals, and widespread use of oral contraceptives could clearly influence population level effects. The analyses of this paper measures the mating patterns of the previous generation, and there is no data concerning whether the parents of the participants of these studies were using oral contraception when they met.

The effect of moderate negative assortative mating on levels of heterozygosity may be so small that the sample sizes were not large enough to reflect them. Other studies have failed to find significant deviation from Hardy-Weinberg equilibrium for class II HLA alleles in a large proportion of populations (e.g. Chen and others 1999; Rivas and others 1997; Soares-Vieira and others 1999). These studies used more powerful tests, such as an exact test using a Markov Chain Monte Carlo approach (Guo and Thompson 1992) and the likelihood ratio test (Weir 1996), although the lack of any pattern in the deviations from expectations among the populations sampled in this paper precludes the need for such power.

Although the HLA region may play an important part in mate choice, a general preference for dissimilar individuals may not best describe its role. Thornhill et al. (2003) found that women prefer the scent of heterozygous individuals as opposed to dissimilar individuals. Jacob et al. (2002) argued that humans might have evolved a desire for an intermediate level of dissimilarity, perhaps through an enhanced effect on immunocompetence, or as a means of minimizing inbreeding and outbreeding costs. Additionally, individuals with particular haplotypes may prefer the scents of individuals with other specific haplotypes. Wedekind (1997) found no evidence that odor preferences aim for particular allelic combinations. Chen et al. (1999) found numerous specific heterozygote frequencies of class II HLA genotypes that were significantly greater or lower than expected among 26 populations, suggestive of particular preferences, although the extremely large number of possible heterozygotic combinations for polymorphic loci increases the possibility for many Type I errors. Although humans' mating preferences may aim for HLA dissimilarity, various haplotypes could emit similar odors that are indiscernible by humans.

If the preference for HLA dissimilar mates is facultative, meaning that it is weighted differently according to its effect on offspring fitness within a particular environment, then individuals living under conditions in which heightened immunocompetence is of greater importance for offspring survival, or perhaps where inbreeding is likely, should display mating patterns in greater accordance with those expected for negative assortative mating along HLA loci. Under similar logic, researchers have found that increased levels of pathogen prevalence are associated with a greater importance placed on attractiveness (Gangestad and Buss 1993), and higher levels of polygyny (Low 1988). The possibility that preference for HLA dissimilar individuals is responsive to environmental conditions is not supported by the fact that Hedrick and Black (1997) found no evidence of negative assortative mating among South Amerindian populations, which inhabit environments with much greater pathogen stress and risk of inbreeding. Even if the preference for HLA dissimilar individuals is not facultative, however, environments in which the importance of other preferences is reduced (e.g. resource potential, willingness to invest, chastity), may lead to a relative increase in the importance of HLA dissimilarity. Similarly, this

preference may be more significant during situations in which the genetic quality of a potential mate is more important, such as in short-term sexual relationships and extra-marital affairs. Women seem to prefer the scent of symmetrical men, but only during high conception risk (Gangestad and Thornhill 1998; Thornhill and Gangestad 1999). Thornhill et al. (2003), however, found no effect of a woman's conception risk with her preference for HLA dissimilar individuals. Mateship studies involving short-term partners would also be very informative, although ethically difficult.

Lastly, HLA dissimilarity may simply not play an important role in human mating patterns. In four odor-preference studies, two unequivocally showed evidence for a preference for the odor of dissimilar individuals; one did so for only one sex, and one showed an opposite effect. These studies have the weakness of not measuring actual mate preferences, but simply odor preferences. The degree to which individuals use this criterion in mate selection is unknown. Among three mateship studies, a more direct test of assortative mating, only one showed a significant effect. The significant effect found among the Hutterites (Ober and others 1997) was complicated by the need to control for a small breeding population with few original ancestors, thereby making interpretation more difficult and possibly prone to error. It is widely known that humans assortatively marry for many attributes, such as attractiveness, religion, socio-economic status, intelligence, stature, various personality traits, and many others (e.g. Jaffe and Chacon-Puignau 1995; Kalmun 1994; Sanchezandres and Mesa 1994). Similarly, men and women have many sex-specific preferences, such as chastity, ambition, or youth (Buss 1989). Along with these criteria, individuals must assess the interest of potential mates in entering into a relationship. All of these factors play roles of varying importance, and although HLA dissimilarity may have a real impact on mate preference, its ultimate effect on marriage patterns may be truly miniscule.

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TABLE 1

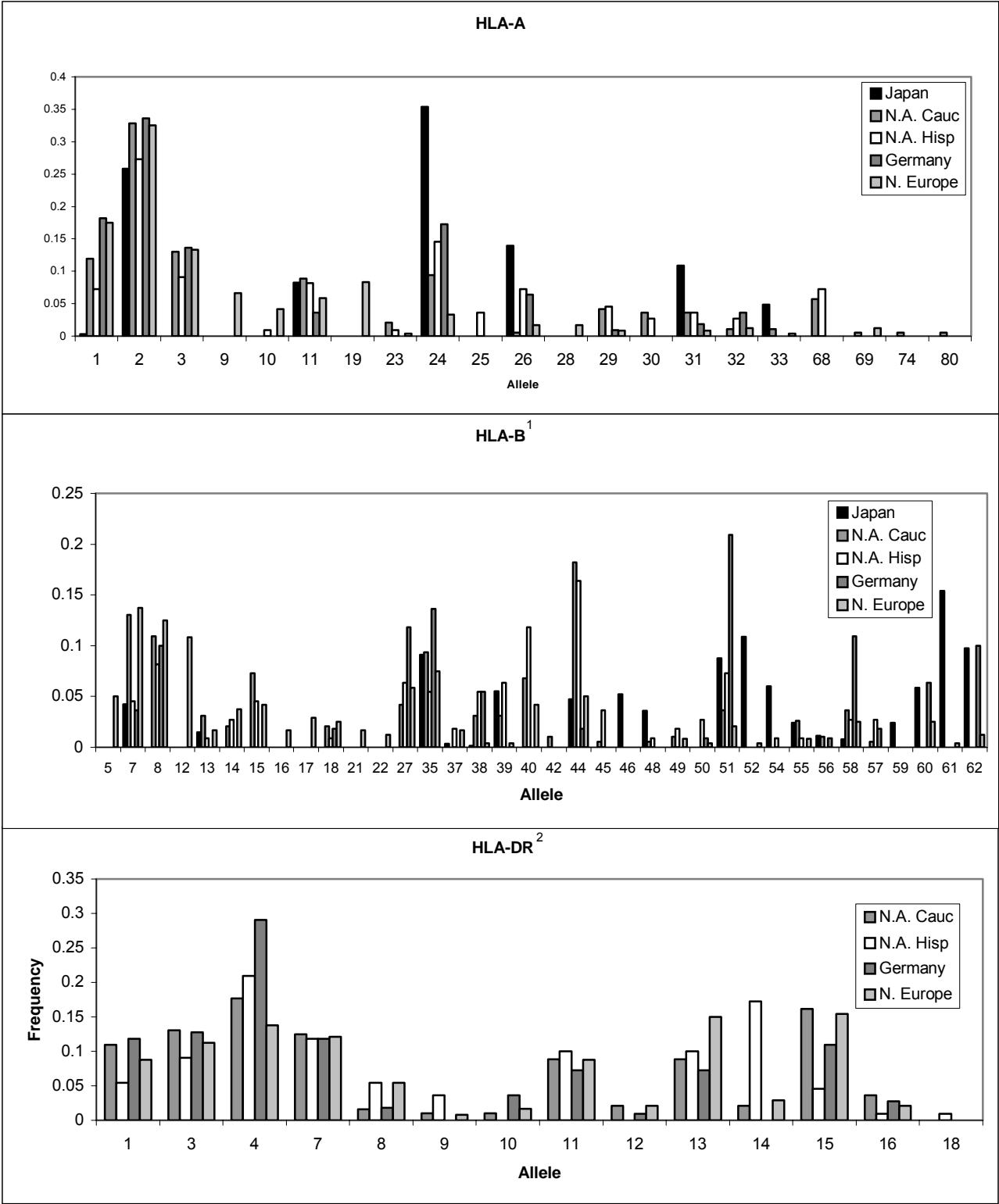
Population	Sample Size	Number of Alleles			Source
		HLA-A	HLA-B	HLA-DR	
Japan	311	7	22	14	1
North America Caucasian	96	16	24	13	2
North America Hispanic	55	14	23	12	2
Germany I	55	9	12	11	3
Germany II	121	16	32	13	4

Source: 1 – Ihara et al. (2000), 2 – Thornhill et al. (2003), 3 – Jacob et al. (2002), 4 – Wedekind and Fürti (1997)

TABLE 2 *Expected and observed frequencies of heterozygotes for the HLA-A, B, and DR loci.*

Population	HLA-A			HLA-B			HLA-DR		
	Exp	Obs	p	Exp	Obs	p	Exp	Obs	p
Japan	0.763	0.768	0.943	0.919	0.903	0.289	0.890	0.877	0.460
North Am Caucasian	0.836	0.896	0.113	0.910	0.885	0.404	0.880	0.906	0.424
North Am Hispanic	0.867	0.873	0.951	0.924	0.982	0.513	0.875	0.891	0.717
Germany I	0.798	0.836	0.481	0.882	0.873	0.825	0.846	0.873	0.587
Germany II	0.827	0.725	0.003	0.923	0.867	0.007	0.887	0.867	0.476

Figure 1 Allelic frequencies for the HLA-A, B and DR loci.



¹ Alleles with frequency less than .01 in all populations not shown

² The Japan dataset utilized different nomenclature and specific allele frequencies are not displayed