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Intensity of natural selection at the major histocompatibility complex loci

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ABSTRACT **Long persistence of allelic lineages, prevalence of nonsynonymous over synonymous substitutions in the peptide-binding region (PBR), and deviation from neutrality of the expected gene identity parameter *F* all indicate indirectly that balancing selection is operating at functional major histocompatibility complex (MHC) loci. Direct demonstrations of the existence of balancing selection at MHC loci are, however, either lacking or not fully convincing. To define the conditions under which balancing selection could be demonstrated, we estimated its intensity from the mean number of nonsynonymous substitutions, K_B, at the PBR and the mutation rate μ. We compared the five available methods for estimating K_B by computer simulation and chose the most reliable ones for estimation of selection intensity. For the human MHC, the selection coefficients of the *HLA-A, -B, -C, -DRB1, -DQB1, -DQA1*, and *-DPB1* loci are 0.015, 0.042, 0.0026, 0.019, 0.0085, 0.0028, and 0.0007, respectively. This low selection intensity places severe restrictions on the possibility of measuring selection directly in vertebrate populations.**

Major histocompatibility complex (MHC) genes encode cell-surface proteins endowed with a peptide-binding region (PBR) that captures short protein fragments and presents them to thymus-derived lymphocytes (1, 2). The MHC–peptide complex is recognized by the T-cell receptor, and the recognition of foreign peptides sets a train of events into motion that leads to a clonal immune response against the parasite from which the peptides are derived. The hallmark of functional MHC genes is their polymorphism, characterized by long persistence of allelic lineages and large number of nucleotide differences between alleles (3, 4). Some of the allelic lineages now found in the human population may have been established as long ago as 85 million years (Myr), before the divergence of prosimians and anthropoid primates (5). Other lineages may be younger, but they all predate the emergence of *Homo sapiens*. The number of nucleotide differences between alleles varies depending on the particular pair of alleles compared but may be as high as 50–60 nucleotides.

The long persistence of allelic lineages suggests the presence of natural selection, since neutral alleles have a relatively short persistence time, of the order of the breeding size of a population (6, 7). Balancing selection is also indicated for the functional MHC genes by the prevalence of nonsynonymous over synonymous substitutions in the PBR (8) and by the deviation from neutrality of expected gene identity (*F*, homozygosity) and linkage disequilibrium (see ref. 9). In spite of these indications, it has proved difficult to demonstrate the presence of balancing selection at MHC loci experimentally. Only a few cases of associations between specific MHC alleles and resistance to parasites in natural populations have been reported (10–12), and even these are not entirely convincing. It is therefore imperative to estimate the intensity of the purported selection pressure and thus define the conditions under which the presence of balancing selection at the MHC loci could be demonstrable. Here, we use a simple model of symmetric overdominant selection (13, 14) and the theory of allelic genealogy (15) to obtain such an estimate.

METHODS AND RESULTS

Estimation of Mutation Rates. Our estimation method requires knowledge both of the mutation rate at the MHC loci and of the effective population size. In the absence of selection, the mutation rate is equal to the nucleotide substitution rate. In the presence of positive (balancing) or negative (purifying) selection, the substitution rate becomes, respectively, higher or lower than the mutation rate (7, 15–18). The functional MHC genes contain regions differentially affected by natural selection: substitutions at the synonymous sites of the coding segment are presumed to be neutral; substitutions at the nonsynonymous sites of the PBR are either deleterious or advantageous; and substitutions at the nonsynonymous non-PBR sites are either deleterious or neutral. Since deleterious mutations do not contribute to the substitution rate (19), we assume that all nonsynonymous substitutions actually observed at the PBR sites are positively selected.

The selection intensity is given by the increase of the substitution rate at the nonsynonymous PBR site over that at the synonymous site. A measure of this increase is a parameter here referred to as γ and defined as the ratio of the number of nonsynonymous substitutions per site to the number of synonymous substitutions per site for a given pair of alleles. In our previous estimates of the synonymous substitution rates at the MHC loci (20, 21), we did not take into account the bias of transitions over transversions that seems to exist at these as well as at other mammalian loci (22–25). If we correct the estimate for this bias, the synonymous substitution rate at the MHC loci becomes 1.0×10^{-9} per site per year. We assume, therefore, that this figure also represents the mutation rate, μ, of the MHC loci, and we use it to calculate u, the mutation rate per PBR per generation, from the formula $u = \mu g L_B$, where g is the generation time and L_B is the number of nonsynonymous PBR sites. Taking $g = 15$ years for primates and $L_B = 115$ for class I loci (26) or $L_B = 50$ for class II loci (27), we obtain $u = 1.7 \times 10^{-6}$ per PBR per generation for class I loci and 7.5×10^{-7} per PBR per generation for class II loci.

Estimation of Nonsynonymous Substitution Rates. The estimator of γ is given by

$$\gamma = \frac{K_B L_S}{K_S L_B}, \qquad [1]$$

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Abbreviations: MHC, major histocompatibility complex; PBR, peptide-binding region; Myr, million years; CV, coefficient of variation.

where K_S and K_B are the mean number of synonymous and nonsynonymous PBR substitutions, respectively, while L_S and L_B are the number of synonymous and nonsynonymous PBR sites, respectively. There are at least five methods of estimating K_B. The most straightforward method is to estimate K_B directly from the available sequences of MHC genes (method I). The problem with this direct approach is that there may be extensive multiple-hit substitutions between distantly related sequences, and it may not be possible to infer these accurately from the observed differences. Another method is to use only the set of genes that are closely related and hence have not experienced extensive multiple-hit substitutions since their divergence from their most recent common ancestor (method II; see ref. 28). The estimator of the mean number of nonsynonymous PBR substitutions is then given by

$$K_B = K_B(m) \div \left[1 - \frac{(m+1)K_S^{m+1}}{(1+K_S)^{m+2} - (1+K_S)K_S^{m+1}}\right], \quad [2]$$

where $K_B(m)$ is the mean number of nonsynonymous PBR substitutions estimated from gene pairs with the number of synonymous substitutions less than m ($K_S \leq m$). The value of m may be chosen as small as 10. An alternative (method III) is to use the linear regression between K_B/L_B and K_S/L_S of pairs of closely related MHC genes. The fourth method of estimating K_B (method IV) is based on the relationship of parameters S and M defined as $S = 2N_e s$ and $M = N_e u$, where N_e is the effective population size and s is the selection coefficient. It has been shown that when S is large and M is small, K_B is approximately given by the number of alleles (n_c) whose frequencies are higher than a certain critical value (15).

$$K_B = n_c. \quad [3]$$

By choosing the critical value of 0.01, n_c is obtained, and this value gives the estimate of K_B. For the use of method IV, the products of the n_c genes must differ by at least one amino acid residue in the PBR. A fifth method can be used when the frequencies of the MHC alleles are known (method V). One can then compute the homozygosity, F, and define its reciprocal as the effective number of alleles, n_e (14). Although in general $n_e < n_c$ the difference becomes small when alleles under strong selection pressure occur in the population at nearly the same frequencies. Hence in method V, the mean number of nonsynonymous PBR substitutions is given by

$$K_B \approx n_e = \frac{1}{F}. \quad [4]$$

Simulation. To compare the accuracy of the K_B-estimation methods, we carried out computer simulations. The assumptions underlying the simulation were these: The population in each generation consists of N_e diploid individuals which mate at random. There are L_B nonsynonymous PBR and L_S synonymous completely linked sites per locus. The mutation rate is $u = \mu g L_B$ per PBR per generation and $v = \mu g L_S$ per synonymous sites of a gene per generation. The state of each site is represented by an integer. Initially, all sites are at state 0, but randomly occurring mutations change the state, each mutation increasing it by one number. This notation system does not allow us to reconstruct the complete genealogy of any allele, but it enables us to determine the number of mutational differences between any two alleles. We used this system to save computer time. We simplified the recording of the genealogy originally used by Takahata and Nei (18) in the following way: Assume two alleles and three sites. The first allele is in states 1, 0, and 2 at sites 1, 2, and 3, respectively; the second allele is in states 0, 1, and 2. The origin of the states at sites 1 and 2 of both alleles is unambiguous, but the state at site 3 could have arisen either by independent mutations in the two alleles or by two mutations occurring at site 3 before the two alleles diverged at sites 1 and 2. We regard the former possibility as unlikely because when we compared, by computer simulation, the number of substitutions that actually were observed during the genealogical process with that inferred from the divergence between the final products of the process, we found the difference not significant. (The inferred numbers were obtained after correction for multiple hits.) Hence, instead of obtaining the actual number of substitutions, we estimated it from the number of differences between the alleles.

Selection was incorporated into the program thus: In each generation we randomly chose pairs of alleles from the pool of $2N_e$ genes. Alleles differing at nonsynonymous PBR sites were passed on to the next generation (heterozygote fitness of $W = 1$). For each pair of alleles identical at these sites a random number R was drawn with values between 0 and 1 and compared with a value of W (homozygote fitness of $W = 1 - s$). In cases of $R \leq W$ a copy of the allelic pair was contributed to the next generation, whereas in cases of $R > W$ it was not; in both cases, however, the alleles were returned to the original pool. This process was repeated until $2N_e$ genes of the next generation were obtained. No recombination was allowed between the selected and the synonymous sites in a gene.

The values of K_B estimated by the different methods are generally in good agreement with one another and with the expected ones (Table 1). Only the K_B values estimated from method III and method IV are slightly larger than expected. Also, the coefficient of variation (CV) in method III is larger than in other methods.

The obtained K_B values were then used to estimate γ. Since γ is known to depend on the mean number of synonymous

Table 1. Five methods for estimating K_B and γ

$M = N_e u$	$N_e s$	Method*	K_B†	γ†
Simulation A				
0.1	100	I	8.5 ± 3.0	3.8 ± 1.2
		II	8.7 ± 2.7	4.2 ± 2.0
		III	11.0 ± 5.4	5.0 ± 3.1
		IV	11.2 ± 1.4	5.7 ± 2.9
		V	9.0 ± 1.0	4.5 ± 2.2
		Expected	8.2	4.2
Simulation B				
0.01	100	I	7.2 ± 4.3	12.4 ± 9.9
		II	6.9 ± 3.1	12.6 ± 9.9
		III	9.7 ± 7.3	13.2 ± 9.6
		IV	7.4 ± 0.8	18.3 ± 22
		V	6.6 ± 0.6	12.5 ± 11
		Expected	6.2	7.5‡

*Methods I–V are based on the following: I, the all-pairwise mean; II, the conditional mean, where the number of synonymous substitutions is less than 10 (see equation 12 in ref. 28); III, the same conditional mean as in method II, but the K_B value is obtained by using the regression coefficient; IV, the number of common alleles with frequencies greater than 0.01; and V, the effective number of alleles.

†The K_B and γ values are based on 20 observations, with each observation made every 8000 and 35,000 generations in simulations A and B, respectively. The N_e was 400 individuals for both simulations. The mutation rates $u = \mu g L_B$ and $v = \mu g L_S$ are assumed to be 2.5×10^{-5} and 1.0×10^{-4} per PBR per generation, respectively, in simulation B. The mutation rates in simulation A were 10 times higher than in simulation B.

‡The discrepancy between the expected and simulated γ values for $M = 0.01$ is attributable to large sampling errors due to small numbers of $N_e u$ and $N_e v$ (25).

substitutions in a pair of alleles of $4N_e\nu$, we tested the reliability of the estimate for two values of $N_e\nu$, 0.04 and 0.4, and obtained mean numbers of synonymous substitutions 3.8 ± 2.6 and 9.9 ± 4.4, respectively. The simulation showed that for the mean number of 3.8, γ fluctuates greatly and the mean is larger than expected, whereas for the mean number of 9.9, very little such skewing occurs (Table 1; see also ref. 25). Hence it is necessary to use only allelic pairs which differ by a sufficiently large number of synonymous substitutions. In the actual data used in the present study, the $N_e\nu$ values are 0.25 or more (see below) and so are suitable for the estimation of γ.

Estimation of Selection Intensity. To estimate the selection coefficient s, we used the relationship

$$s = \frac{S}{2N_e}, \quad [5]$$

where

$$S = \frac{K_B^2\gamma}{\sqrt{2}} \quad [6]$$

and

$$N_e = \frac{M}{u}, \quad [7]$$

whereby

$$M = \sqrt{\frac{S}{16\pi}}\exp\left(-\frac{\gamma}{\sqrt{2}}\right). \quad [8]$$

Table 2 gives the estimates of K_B, γ, S, and M for seven functional MHC loci: *HLA* class I *A*, *B*, and *C*, as well as class II *DRB1*, *DQB1*, *DPB1*, and *DQA1*. Most K_B estimates based on methods I, IV, and V are smaller than those based on methods II and III. The difficulty inherent in method I is to make an accurate multiple-hit correction of K_B between distantly related alleles. In fact, a plot of the number of nonsynonymous PBR substitutions (even after multiple-hit correction by conventional methods) against the number of synonymous substitutions indicates that the former does not increase linearly with the latter (28).

The allele frequency data used in methods IV and V are derived from serological typing, which does not distinguish all the alleles. Thus, for example, of the six *HLA-A2* alleles differentiated at the nucleotide level, only three are distinguishable serologically (30). Moreover, the so-called "blanks" (allomorphs that do not react with any of the available allomorph-specific serological reagents) may consist of several alleles distinguishable at the nucleotide level. Because of these two inadequacies, methods IV and V tend to underestimate the K_B and γ values, while methods II and III give more reliable results.

For any of the five methods, the CV of K_B is relatively small, but the CV of γ is large if the number of synonymous substitutions or the values of $N_e\nu$ are small (Eq. 1 and Table 1). Long stretches of DNA sequence must therefore be compared to obtain reliable estimates of γ. For this reason, we could not use many of the partial sequences available. For the entire coding region, the observed mean number of synonymous substitutions at each of the MHC loci is about 10, and therefore the expected value of $N_e\nu$ is approximately 0.25. Under these conditions, there should be no serious skewing in the distribution of γ (simulation A in Table 1).

As pointed out above, the effective population size, N_e, can be inferred from the estimated M and u values. The estimates of M for the class I loci *A*, *B*, and *C* are 0.09, 0.02, and 0.29, respectively, the average being 0.13. The estimates of M for the class II loci *DRB*, *DQB*, *DPB*, and *DQA* are 0.01, 0.08, 0.08, and 0.14, respectively, with an average of 0.08. From the estimated values of u for class I and class II loci, we obtain N_e values of $(0.8 \pm 0.6) \times 10^5$ and $(1.1 \pm 0.6) \times 10^5$, respectively, with an average of approximately 10^5.

There appear to be differences in the intensity of selection operating at different *HLA* loci (Table 3). The strongest selection is operating at the *HLA-B* locus, followed by *-DRB1*, and *-A* loci; weak selection is operating at *HLA-DQB*, *-DQA*, *-C*, and *-DPB1* loci. These differences in selection intensity correlate well with differences in the degree of polymorphism among the different *HLA* loci and with the trans-specific retention of the polymorphism (30–35). The *HLA-B* and *-DRB1* loci are more polymorphic than the remaining *HLA* loci and their alleles are members of very ancient lineages. Some of the lineages at the *DRB1* locus, for

Table 2. Estimates of the mean number of nonsynonymous substitutions and the relative nonsynonymous substitution rate (γ) in the PBR (K_B)

Method	*HLA* locus	K_B	γ	S	M
I	*A*	16	3.8	690	0.25
	B	19	4.7	1200	0.18
	C	8.4	1.9	(95)	0.36
	DRB1	15	5.9	990	0.07
	DQB1	12	3.5	360	0.22
	DPB1	4.3	2.7	(35)	0.12
	DQA1	5.8	2.0	(48)	0.23
II	*A*	26	6.3	3000	0.09
	B	36	9.0	8200	0.02
	C	15	3.4	530	0.29
	DRB1	25	9.3	3900	0.01
	DQB1	20	6.0	1700	0.08
	DPB1	6.8	4.3	140	0.08
	DQA1	13	4.5	550	0.14
III	*A*	23	5.5	2100	0.12
	B	24	6.1	2500	0.09
	C	8.8	2.0	(110)	0.36
	DRB1	17.8	6.8	1500	0.05
	DQB1	14	4.1	570	0.18
	DPB1	4.3	2.7	(35)	0.12
	DQA1	8.3	2.8	140	0.22
IV*	*A*	16	3.8	690	0.25
	B	28	6.9	3800	0.07
	C	11	2.5	210	0.36
	DRB1	15	5.9	990	0.07
V*	*A*	8.8	2.1	(110)	0.36
	B	22	5.7	2000	0.10
	C	5.8	1.3	(31)	0.31
	DRB1	13	4.7	560	0.12

The values in parentheses do not satisfy the assumption $\sqrt{S}/M \geq 100$ of the theory of allelic genealogy (15).
*Allele frequency data are taken from ref. 29; frequencies of *DQB1*, *DPB1*, and *DQA1* alleles were not available.

Table 3. Estimated selection coefficient (s) based on method II

HLA locus	s, %
A	1.5
B	4.2
C	0.26
DRB1	1.9
DQB1	0.85
DPB1	0.07
DQA1	0.28

The long-term breeding size in the human lineage N_e is estimated as approximately 10^5 (see text; ref. 28).

example, have recently been traced to the common ancestors of prosimians and anthropoid primates who lived some 85 Myr ago (5). This correlation makes sense, since the lineages could not have persisted for such a long time without selection. At the *HLA-B* locus, sharing of allelic lineages with other primates is not so clear as that at the *-DRB1* locus (36–38). The large number of synonymous substitutions accumulated among *HLA-B* alleles, however, shows that the mean divergence time of alleles at this locus is more than 15 Myr, and the longest divergence time is more than 30 Myr. Such a long persistence time is also consistent with strong selection operating at this locus.

DISCUSSION

Selection operating at MHC loci is often described as strong, without "strong" being precisely defined. The estimation methods used in the present study indicate that even at the *HLA-B* locus, which appears to be under the strongest selection pressure, the selection coefficient is <5%. Although selection which effects more than 1% difference in viability can significantly influence the fate of a gene on an evolutionary time scale, it in fact places severe limitations on attempts to estimate the intensity of selection experimentally or by observation. Here, we consider the possibility of detecting changes in genotype as well as gene frequencies caused by viability selection (see refs. 39–41 for detecting other types of selection).

We studied two estimators of a selection coefficient. Let H and F be the proportions of hetero- and homozygotes before selection in generation 1 (where $H + F = 1$), and denote their relative viability by 1 and $1 - s$, respectively. After selection, the proportion of heterozygotes H' becomes $H/(1 - sF)$. Hence by determining the genotype frequencies, we obtain $S_1 = (H' - H)/(H'F)$ as an estimator of s. Alternatively, we can use the gene frequency change through generations for estimating s. If we denote the frequency of the ith allele at the tth generation by $p_i(t)$, the increment of $p_i(t)$ in a generation is given by $\Delta p_i(t) = sp_i(t)[F - p_i(t)]/(1 - F)$. The second estimator of s is then given by $S_2 = \Delta p_i(t)/[p_i(t + 1)F - \{p_i(t)\}^2]$.

In an actual population, all gene and genotype frequencies can be subjected to genetic drift. The smaller the population, the larger the effect of genetic drift. In addition, gene and genotype frequencies are estimated by taking a sample from a population, and the sampling may obscure the effect of selection unless the sample size is sufficiently large. Thus, the reliability of the estimate of s depends not only on its magnitude but also on the number (N_e) of breeding individuals in the population and the sample size (n). To examine the statistical properties of S_1 and S_2, we conducted computer simulations. We assumed that there are only two segregating alleles in a population and that the size ($N_e = 10^4$) is large enough for selection to predominate ($s = 0.05$). For each set of parameter values, we repeated the simulation 10^4 times and computed the probability (P) that S_1 or S_2 lies within ± 10% of the true s value. The initial allele frequency [$p(0)$] of one of the two alleles was set as 0.01 or 0.2. Since selection is less efficient for rare alleles, neither the S_1 nor the S_2 estimator worked well for small $p(0)$. If the population is at equilibrium, S_2 cannot be used. A third estimator, S_3, based on the reduced gene frequency variance, could be developed for the equilibrium condition. However, our simulation shows that the reduction in variance induced by selection is small and requires large sample size as well as the passage of several generations to allow reliable estimates of selection.

A statistically significant P value for S_1 and S_2 estimates can be attained only when n is large (at least 1000). In this case, S_1 is a slightly better estimator than S_2: For $n = 1000$, P equals 0.05 for the S_1 estimator, whereas it equals 0.033 when the S_2-based method is used. For $n = 9000$, the S_1-based P value increases to 0.18. Nevertheless, this value is still too small to make reliable estimates of s.

When there are more than two alleles segregating in a population, the detection of selection becomes even more difficult. As the number of alleles (k) increases, the F value becomes smaller. The reduction of F is inversely proportional to k, so that selection becomes as weak as $s/(k - 1)$. Since there are more than 10 alleles at any of the functional MHC loci in most vertebrate populations, the frequency data from a natural population may not be suitable for estimating the selection intensity.

The prospects are somewhat improved when observations are made over successive generations. Suppose that we take the arithmetic mean of each estimator based on observations over t generations. The P value can then be high. For example, if $s = 0.05$, $n = 5000$, $p(0) = 0.2$, and $t = 20$, then $P = 0.47$ for S_1 estimator (cf. $P = 0.12$ with $t = 1$) and the 90% confidence interval of the estimated s ranges from 0.035 to 0.060. We may note, however, that the required condition is not realistic for most vertebrate species.

It could be argued that the estimate of <5% is an average selection coefficient and that virulent or new parasites may exert a stronger, experimentally measurable, selection pressure. *Plasmodium falciparum* is one of the most devastating human parasites. In the population, however, in which positive association between malaria and MHC has been reported, the selection coefficient with respect to the MHC genes has been estimated to be of the same order as that given in our own calculations (42).

If a selection coefficient of less than 0.05 is sufficient to maintain polymorphism for long periods of time, then selection at other loci, which do not show any sign of trans-specific polymorphism, must be much weaker than that of the MHC loci. And if it is so difficult to estimate the intensity of balancing selection at the MHC loci from frequency data, it will be even more difficult, if not impossible, at other loci. In such cases, indirect evidence based on DNA sequence differences may be the only possibility for obtaining reliable estimates of selection.

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1. Klein, J. (1986) *Natural History of the Major Histocompatibility Complex* (Wiley, New York).
2. Bjorkman, P. J. & Parham, P. (1990) *Annu. Rev. Biochem.* **59**, 253–288.
3. Klein, J. & Figueroa, F. (1986) *Nature (London)* **329**, 512–518.
4. Klein, J., Satta, Y., O'hUigin, C. & Takahata, N. (1993) *Annu. Rev. Immunol.* **11**, 269–295.
5. Figueroa, F., O'hUigin, C., Tichy, H. & Klein, J. (1994) *J. Immunol.* **152**, 4455–4465.
6. Kimura, M. (1967) *Genet. Res.* **9**, 23–24.
7. Kimura, M. (1983) *The Neutral Theory of Molecular Evolution* (Cambridge Univ. Press, Cambridge, U.K.).
8. Hughes, A. L. & Nei, M. (1988) *Nature (London)* **335**, 167–170.
9. Klitz, W., Thomson, G. & Baur, M. P. (1986) *Am. J. Hum. Genet.* **39**, 340–349.
10. De Vries, R. R. P., Khan, P. M., Bernini, L. F., van Loghem, E. & van Rood, J. J. (1979) *J. Immunogenet.* **6**, 271–287.
11. Briles, W. E., Stone, H. A. & Cole, R. K. (1977) *Science* **195**, 193–195.
12. Hill, S. A., Allsopp, C. E. M., Kwiatkowski, D., Anstey, N. M., Twumasi, P., Rowe, P. A., Bennett, S., Brewster, D., McMichael, A. J. & Greenwood, B. M. (1991) *Nature (London)* **352**, 595–600.
13. Wright, S. (1939) *Genetics* **24**, 538–552.
14. Kimura, M. & Crow, J. F. (1964) *Genetics* **49**, 725–738.
15. Takahata, N. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2419–2423.
16. Kimura, M. (1968) *Nature (London)* **217**, 624–626.
17. Maruyama, T. & Nei, M. (1981) *Genetics* **98**, 441–459.

18. Takahata, N. & Nei, M. (1990) *Genetics* **124,** 967–978.
19. Kimura, M. (1991) *Jpn. J. Genet.* **66,** 367–386.
20. Satta, Y., Takahata, N., Schönbach, C., Gutknecht, J. & Klein, J. (1991) in *Molecular Evolution of the Major Histocompatibility Complex*, eds. Klein, J. & Klein, D. (Springer, Heidelberg), pp. 51–62.
21. Satta, Y., O'hUigin, C., Takahata, N. & Klein, J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 7480–7484.
22. Li, W.-H. (1993) *J. Mol. Evol.* **36,** 96–99.
23. Pamilo, P. & Bianchi, N. O. (1993) *Mol. Biol. Evol.* **10,** 271–280.
24. Kondo, R., Horai, S., Satta, Y. & Takahata, N. (1993) *J. Mol. Evol.* **36,** 517–531.
25. Satta, Y. (1993) *Immunogenetics* **38,** 450–454.
26. Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987) *Nature (London)* **329,** 512–518.
27. Brown, J. H., Jardetzky, T. S., Gorga, J. C., Stern, L. J., Urban, R. G., Strominger, J. L. & Wiley, D. C. (1993) *Nature (London)* **364,** 33–39.
28. Takahata, N., Satta, Y. & Klein, J. (1992) *Genetics* **130,** 925–938.
29. Central Data Analysis Committee (1991) *The Data Book*, 11th International Histocompatibility Workshop (Central Data Analysis Committee, Yokohama, Japan), Vol. 2.
30. The WHO Nomenclature Committee for Factors of the HLA System (1992) *Immunogenetics* **36,** 135–148.
31. Watkins, D. I., Zemmour, J. & Parham, P. (1993) *Immunogenetics* **37,** 317–330.
32. O'hUigin, C., Bontrop, R. E. & Klein, J. (1993) *Immunogenetics* **38,** 165–183.
33. Zemmour, J. & Parham, P. (1993) *Immunogenetics* **37,** 239–250.
34. Marsh, S. G. E. & Bodmer, J. G. (1993) *Immunogenetics* **37,** 79–94.
35. Bontrop, R. E. (1994) *Immunogenetics* **39,** 81–92.
36. Mayer, W. E., Jonker, M., Klein, D., Ivanyi, P., van Seventer, G. & Klein, J. (1988) *EMBO J.* **7,** 2765–2774.
37. Lawlor, D. A., Warren, E., Taylor, P. & Parham, P. (1991) *J. Exp. Med.* **174,** 1491–1509.
38. Hughes, A. L., Hughes, M. K. & Watkins, D. I. (1993) *Genetics* **133,** 669–680.
39. Prout, T. (1971) *Genetics* **63,** 949–967.
40. Christiansen, F. B., Frydenberg, O. & Simmonsen, V. (1973) *Hereditas* **73,** 291–304.
41. Clark, A. G. & Feldman, M. W. (1981) *Heredity* **46,** 347–377.
42. Hill, A. V. S. (1991) in *Molecular Evolution of the Major Histocompatibility Complex*, eds. Klein, J. & Klein, D. (Springer, Heidelberg), pp. 403–420.