# POSSIBLE *MHC* ASSOCIATED HETEROZYGOUS ADVANTAGE IN WILD MOUSE POPULATIONS

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*The variability of the major histocompatibility complex (Mhc) in natural populations is usually very extensive, but the forces that are responsible for the maintenance of this variability are still unknown. Several investigators (Hughes and Nei 1988, 1989; Jonsson et al. 1989; Takahata and Nei 1990) have recently suggested that the polymorphism in the region of the peptide-binding sites is under positive selection. A verification of this suggestion should be obtained by empirical studies of natural populations, and a good model animal for such studies is the house mouse (Mus domesticus and M. musculus), for which a great deal of information is available both as far as the structure and dynamics of natural populations (Berry 1981) and the organization of H-2, its major histocompatibility complex (Klein 1986), is concerned. A problem associated with the demonstration of the effect of selection on H-2 variability, however, is the fact that in general, natural populations of the house mouse are highly polymorphic for H-2 haplotypes (Klein and Figueroa 1986), so that all individuals are expected to be heterozygotes, even without selection (Duncan et al. 1979). The discovery of low H-2 variability in two adjacent populations of the house mouse (M. domesticus) in Jerusalem, Israel (Neufeld et al. 1986), in which some individuals were shown to be homozygotes, provided a good opportunity to find out whether H-2 heterozygotes have a selective advantage.*

The two mouse populations live in the Educational Farm of the Hebrew University in Jerusalem. One ("Population I") occupies an active chicken coop, the area of which is 100 $m^2$. The other ("Population II") lives in an adjacent, 250 $m^2$ field, in which 20 permanent "mouse stations" (containing a constant supply of food and water) were established. Both populations are unconfined and no mouse was artificially introduced into them.

The dynamics of the two populations was followed by bi-weekly (each of two successive nights) or monthly (each of four successive nights) trapping series. The survey began in November 1982 and continued until October 1985. The survey in 1983 was used to characterize the different *H-2* haplotypes found in the populations. It was discovered (Neufeld et al. 1986) that there are only four haplotypes in the two populations - *H-2*$^{w82}$

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($K^{w16}D^{w82}$), *H-2*$^{w83}$ ($K^{w83}D^{w16}$), *H-2*$^{w84}$ ($K^{w84}D^{w84}$) and *H-2*$^{w85}$ ($K^{w83}D^{w84}$). *H-2*$^{w85}$ is a recombinant between *H-2*$^{w83}$ and *H-2*$^{w84}$. The antisera prepared against these four haplotypes were anti-$K^{w16}$, anti-$D^{w82}$, anti-$K^{w83}$, and anti-*H-2*$^{w84}$.

The analysis of all the mice that belonged to the two populations, using the four antisera, began in June 1984 (trapping series 36) and ended in October 1985 (trapping series 54). This period seems to cover, in both populations, a full cycle of population size variation.

**Table 1.** *H-2* genotypes deduced from the results of the cytotoxic test.

| Reactivity with antisera specific for | | | | Deduced genotype |
|---|---|---|---|---|
| *Kw16* | *Dw82* | *Kw83* | *H-2w84* | |
| + | + | - | - | *H-2w82/H-2w82* |
| - | - | + | - | *H-2w83/H-2w83* |
| - | - | - | + | *H-2w84/H-2w84* |
| + | + | + | - | *H-2w82/H-2w83* |
| + | + | - | + | *H-2w82/H-2w84* |
| + | + | + | + | *H-2w82/H-2w85* |
| - | - | + | + | *H-2w83/H-2w84* |
| | | | or | *H-2w83/H-2w85* |
| | | | or | *H-2w84/H-2w85* |
| | | | or | *H-2w85/H-2w85* |

+, positive; -, negative

Adult mice (15 g or more) were temporarily taken to the laboratory and one lymph node was removed from each. The *H-2* genotype of a mouse was determined by the reactivity of its lymphocytes with the four antisera, using the cytotoxic test. Table 1 gives the deduced genotypes, according to the different patterns of reactivity. One pattern (negative reactions with anti-$K^{w16}$ and $D^{w82}$, positive reactions with anti-$K^{w83}$ and anti-*H-2*$^{w84}$) can represent more than a single genotype. No new haplotype was discovered, and the existence of homozygotes was confirmed by matings. A sporadic survey was carried out in Population I in 1989-1990, using antisera that could identify unambiguously all genotypes.

Tables 2 and 3 give the distribution of the different *H-2* genotypes in the different series, separately for Population I (Table 2) and II (Table 3). For each series, the tables include population size, the number of mice for which the *H-2* genotypes were known, and the number of mice that had each genotype.

**Table 2.** Number of mice with different *H-2* genotypes in Population I.

| Series number | Population size | Number of mice with known genotype | Mice with genotype | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *82/82* | *83/83* | *84/84* | *82/83* | *82/84* | *82/85* | *83/84/85* |
| 36 | 12 | 7 | | | 3 | | 2 | | 2 |
| 37 | 14 | 8 | | 1 | 3 | | 2 | | 2 |
| 38 | 12 | 9 | | 1 | 3 | 1 | 3 | | 2 |
| 39 | 16 | 12 | | 1 | 3 | 1 | 2 | 2 | 3 |
| 40 | 15 | 14 | 1 | 1 | 3 | 4 | 3 | 2 | 3 |
| 41 | 20 | 18 | | 2 | 2 | 3 | 3 | 5 | 2 |
| 42 | 23 | 20 | | 4 | 1 | 3 | 3 | 6 | 3 |
| 43 | 23 | 20 | | 4 | 1 | 3 | 2 | 8 | 2 |
| 44 | 27 | 23 | | 4 | 1 | 4 | 1 | 10 | 4 |
| 45 | 36 | 28 | | 4 | 1 | 5 | 2 | 10 | 7 |
| 46 | 53 | 40 | | 4 | 1 | 4 | 4 | 18 | 8 |
| 47 | 49 | 37 | | 4 | 1 | | 3 | 17 | 8 |
| 48 | 38 | 28 | | 1 | 1 | | 2 | 16 | 8 |
| 49 | 39 | 29 | | 1 | 1 | | 2 | 19 | 7 |
| 50 | 36 | 30 | | | | | 1 | 17 | 12 |
| 51 | 29 | 20 | | | | | 1 | 12 | 7 |
| 52 | 13 | 10 | | | | | | 8 | 2 |
| 53 | 13 | 11 | | | | | | 7 | 4 |
| 54 | 7 | 6 | | | | | | 3 | 3 |

At the beginning, when the size of Population I was small, *H-2* homozygotes were relatively common (Table 2). With time (as population size increased), the homozygotes became less frequent (with *H-2w83*/*H-2w83* replacing *H-2w84*/*H-2w84*) and disappeared completely from Population I after series 49. They did not even reappear when population size was reduced. Another change was an increase in the proportion of genotypes *H-2w82*/*H-2w85*, or of mice with haplotype *H-2w85*.

**Table 3.** Number of mice with different *H-2* genotypes in Population II.

| Series number | Population size | Number of mice with known genotype | Mice with genotype | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *82/82* | *83/83* | *84/84* | *82/83* | *82/84* | *82/85* | *83/84/85* |
| 36 | 17 | 5 | | | 1 | | 2 | 1 | 1 |
| 37 | 13 | 6 | | | 1 | | 2 | 1 | 2 |
| 38 | 10 | 8 | | | 1 | | 3 | 1 | 3 |
| 39 | 12 | 10 | | | 1 | | 5 | 1 | 3 |
| 40 | 12 | 11 | | | 2 | | 6 | 1 | 2 |
| 41 | 7 | 6 | | | 1 | | 3 | | 2 |
| 42 | 8 | 8 | | | 1 | | 4 | 1 | 2 |
| 43 | 16 | 14 | | 1 | 1 | | 5 | 5 | 2 |
| 44 | 22 | 15 | | 2 | 1 | | 2 | 8 | 2 |
| 45 | 19 | 16 | | 2 | 1 | | 3 | 8 | 2 |
| 46 | 24 | 21 | | 3 | 4 | | 4 | 8 | 2 |
| 47 | 32 | 27 | | 3 | 4 | 2 | 3 | 13 | 2 |
| 48 | 29 | 25 | | 2 | 1 | 2 | 3 | 15 | 2 |
| 49 | 28 | 27 | | 4 | 1 | 2 | 1 | 16 | 3 |
| 50 | 23 | 20 | | 4 | 2 | 2 | 1 | 9 | 2 |
| 51 | 17 | 15 | | 2 | | 2 | 1 | 7 | 3 |
| 52 | 15 | 12 | | 1 | | 1 | | 8 | 2 |
| 53 | 10 | 10 | | 1 | | 1 | | 6 | 2 |
| 54 | 12 | 10 | | 1 | | 1 | | 5 | 3 |

Similar changes (although less extreme) were evident in Population II (Table 3). An analysis of 35 mice from Population I in October 1989 (nine mice), February 1990 (11 mice), and May 1990 (15 mice) indicated that it continued to be composed exclusively of mice that have the same four haplotypes. The proportions of *H-2w82*, *H-2w84*, and *H-2w85* haplotypes were almost equal (each haplotype occurred at a frequency of about 30%), while the proportion of haplotype *H-2w83* was about 10%. As in the larger survey, the *H-2* homozygotes (*H-2w84/H-2w84* and *H-2w85/H-2w85*) were more common (33%) when population size was small (October 1989) and less common when population size increased (17% in February 1990, 13% in May 1990).

Can the changes in the frequencies of the different genotypes be caused by random events? A major problem with the statistical analysis of our results is the overlap between

the series with regard to the mice that were captured in each. To overcome this problem we decided to consider each mouse only once - either at the series of its first capture or, for a second comparison, at the series of its last capture.

For the statistical analysis we combined the mice from both populations. In each series the mice were divided into two categories - homozygotes (*H-2$^{w82}$/H-2$^{w82}$*, *H-2$^{w83}$/H-2$^{w83}$*, and *H-2$^{w84}$/H-2$^{w84}$*) and heterozygotes *H-2$^{w82}$/H-2$^{w83}$*, *H-2$^{w82}$/H-2$^{w84}$*, and *H-2$^{w82}$/H-2$^{w85}$*). Mice listed in Tables 2 and 3 in column 83/84/85 were excluded. If there is no selective difference between homozygotes and heterozygotes, they should join the population, or disappear from it, at random. To test this hypothesis we used the One-Sided Wilcoxon Rank Sum Test (Ostle 1963). All mice were ranked according to the time of their first (or last) appearance, and the positions of all members in each category were summed. The difference, T, between the two sums was used to calculate *t*.

For the time of first appearance, the sum of ranks was 940 for homozygotes and 7316 for heterozygotes. The value of *t* (2.6670) is highly significant. In the analysis of discontinuing mice we reached a figure of 1089 for homozygotes and 7617 for heterozygotes, again giving a significant value of *t* (-1.7083). We conclude that the average time of appearance and disappearance of *H-2* homozygotes is significantly shorter than that of heterozygotes, suggesting the operation of selection in favor of heterozygotes.

A similar calculation was carried out for mice divided into a category that included all animals with at least one copy of haplotype *H-2$^{w85}$*, and a category of all other animals. In contrast to the first statistical test, which excluded some mice, this test included all mice. For the time of first appearance, the sums of ranks were 11172 for mice with *H-2$^{w85}$* and 2689 for mice without *H-2$^{w85}$*. The *t* value (5.7551) is highly significant. For discontinuing mice the sums of ranks were 10963 (with *H-2$^{w85}$*) and 2898 (without *H-2$^{w85}$*), again giving a highly significant *t* value (5.0274). Selection seems to be favoring mice with haplotype *H-2$^{w85}$*.

A simulation study, based on an initial population with a composition similar to that of Population I at the beginning of the survey, and continued for four additional generations (in which population sizes were 5, 21, 15, and 9 mice), showed that in 2000 independent runs, the probability of increasing by chance (without selection) the proportion of heterozygotes, or of mice with *H-2$^{w85}$* to the level found at the end of our survey, was close to zero (Ritte et al. 1991).

*H-2* homozygotes become more common when the population undergoes major declines, usually at the end of the summer. When population size increases, *H-2* homozygotes are selected against. In our particular population, the favored genotype was *H-2$^{w82}$/H-2$^{w85}$*.

Among homozygotes, the proportion of each genotype is often much lower than the square of the proportion of its haplotype. This is true especially for the genotype *H-2$^{w82}$/H-*

$2^{w82}$ which was found only in one individual, although the haplotype $H\text{-}2^{w82}$ was relatively common.

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