these being classified as nonhomologous or unacceptable replacements. The acceptability or unacceptability of mutation pairs is quite understandable on the basis of the chemical similarities or dissimilarities existing between the members of each pair.

Although the inclusion of these acceptable or conservative replacements is certainly necessary in any evaluation of homology, it must be kept in mind that the highest emphasis must always be given to the number of identical residues.

In Fig. 1 are shown the segments of the LTH and HGH sequences having clearly demonstrable homology. The longest of these include the segments containing the 43 COOH-terminal residues from these two molecules. In order to align these two segments, gaps must be introduced into the HGH structure. Addition of these gaps results in a total of 45 residue positions. The homology between the segments consists of 14 pairs of identical residues, 14 pairs of highly acceptable mutations, and 7 pairs of acceptable mutations. Thus, 35 of the 45 residue positions show a high degree of homology. It may be significant that in the segments of HGH and LTH shown in Fig. 1 the homology is poorest adjacent to the positions where a gap has been introduced.

Additional regions of good homology are found near the middles of the two sequences. Figure 1 also shows the alignment from Cys-58 of LTH and Cys-68 of HGH. The comparison is quite good up to Leu-80 of LTH and Leu-90 of HGH. At this point a repositioning of the HGH chain is required to continue fitting to the LTH structure. If Ile-17 of HGH is aligned with Met-81 of LTH the homology may be extended for an additional 14 residue pairs. This is an important alignment since it identifies which of the two tryptophan residues in LTH (Trp-90) is homologous with the single tryptophan (Trp-25) in HGH. Final areas of homology are shown in Fig. 1, beginning with Ala-22 of LTH and Pro-28 of HGH and extending for 23 residue pairs.

The remaining two portions of each molecule do not show such a high degree of homology. In particular, the content of identical residues is considerably lower. These last two alignments are tabulated in Table 1.

Thus, three areas of these two hormone molecules appear to be homologous in terms of their primary structure (Table 1). These areas contain a total of 105 residues of which 35 are identical, 36 are highly acceptable replacements, and 13 are acceptable replacements giving a total of 84 homologous positions. This amounts to about 45 percent of either peptide chain. There appears to be sufficient similarity in the sequences of LTH and HGH to confidently postulate their separate evolution from a common ancestor molecule.

Since HGH is active both as a lactogenic and a growth hormone it is highly probable that some portion or portions of the three areas showing the highest degrees of homology contain important structural features intrinsically required for lactogenic activity. Of course, some or all of these same features may also be required for growth hormone activity, and we are not proposing that the two activities reside in mutually exclusive portions of the HGH molecule. It may be that these two biological functions are controlled by a single basic mechanism and that the real difference in the two molecules is in their specificity for receptors.

At the present time the significance of an evolutionary link between these two hormones in terms of their mechanisms of action is unknown. Nevertheless, the acceptance of such a link may provide directions along which useful theories may be made and ultimately tested in the laboratory.

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4. Abbreviations for amino acid residues are as follows. Alanine, Ala; arginine, Arg; aspartic acid, Asp; asparagine, Asn; cysteine, Cys; glutamic acid, Glu; glutamine, Gln; glycine, Gly; histidine, His; isoleucine, Ile; leucine, Leu; lysine, Lys; methionine, Met; phenylalanine, Phe; proline, Pro; serine, Ser; threonine, Thr; tryptophan, Trp; Tyrosine, Tyr; and valine, Val.
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Histocompatibility-2 (H-2) Polymorphism in Wild Mice

Abstract. *Red blood cells of 40 wild mice captured at four different locations in the Ann Arbor area were typed for the presence of 15 different H-2 antigens by direct hemagglutination test. Fifteen different phenotypes were found and all were different from those determined by known* H-2 *alleles of the laboratory mice. Great similarity between phenotypes of mice from the same location was observed. This is interpreted as further evidence for the deme structure of natural mouse populations.*

The major histocompatibility locus of the house mouse, *Mus musculus*, is *H-2*. Its products are the most potent antigens of tissue incompatibility and, at the same time, antigens of the most complex blood group system of this species. The complexity of the *H-2* locus is best illustrated by the so-called H-2 chart (*1*) which lists about 25 well-defined H-2 antigens in about 20 well-defined combinations (*H-2* alleles), according to their distribution in various inbred strains and stocks. Since most of the strains and stocks of the laboratory mouse were derived from a very small number of sources, the H-2 chart does not give a true picture of the H-2 polymorphism. To get information about the extent of polymorphism in natural populations, one has to study wild mice. In this report preliminary data on such analyses are presented.

The wild mice were trapped in four farms in the Ann Arbor area during the summer of 1969. The farms were 10 km or more apart and 5 km or more beyond the city limits. The mice were captured either in granaries (farms KP, KE, and SA) or in corncribs (farms KE and GA). H-2 antigens were identified with alloantiserums, most of them prepared by immunization of F_1 hybrids between two inbred strains with tissue from genetically different inbred strains. The donor-recipient combinations were chosen in such a way as to produce monospecific or nearly monospecific antiserums (*2*). Prior to their employment in the wild mice study, all the antiserums were analyzed serologically with a panel of red blood cells representing different *H-2* alleles. A total of

23 different antiserums were used, which allowed us to test the presence or absence of 15 different H-2 antigens. The antiserums are listed in Table 1.

The hemagglutination tests were performed in polyvinyl-pyrrolidone as described by Stimpfling (*3*), with modifications introduced by Shreffler (*4*). Each antiserum was tested at least three times with the red blood cells of each mouse. In 92 percent of the tests all the repeats gave a concordant result. Conclusions on the presence or absence of an antigen were based on at least two out of three concordant results.

The results of typing of 40 wild mice from four different locations for the presence of 15 different H-2 antigens are summarized in Fig. 1. In this sample of the wild population of *Mus musculus*, 15 different combinations of H-2 antigens were found. None of them was identical with any of the 20 *H-2* alleles present in the laboratory strains and stocks of mice. However, some of the wild mice are undoubtedly H-2 heterozygotes and the number of different *H-2* alleles in the sample could be lower than the number of detected H-2 phenotypes. Evidence for the existence of several previously undescribed H-2 phenotypes was also presented by Rubinstein and Ferrebee (*5*) in a study of random-bred Swiss-Webster mice, and by Ivanyi (*6*). It is, therefore, obvious that the degree of polymorphism of the *H-2* locus in the mouse population is considerably greater than is indicated in the present H-2 chart of the laboratory mouse.

Mice captured at the same location had conspicuously similar H-2 phenotypes. Thus, for instance, all the mice from farm KP carried antigen H-2.17, which was absent in the mice from the other three farms. On the other hand, mice from farm KE carried antigen H-2.16 (and some of them also H-2.9) which was again absent in the mice from the other locations. Some mice from farm GA carried only antigen H-2.5 while the other 14 antigens were absent. Others from the same farm lacked all 15 antigens. All this suggests that the natural population of the house mouse is composed of breeding units with a high rate of inbreeding within the units. The fractionation of the natural mouse population into small, relatively isolated, endogamous family units (demes) was first postulated by Lewontin and Dunn (*7*). Empirical evidence for the existence of such breeding structure was presented by Anderson (*8*) and Petras (*9*). The H-2 data presented here provide further support for this concept. Apparently in at least three of the four locations (farms SA, GA, and KP) the trapping area was occupied by only one deme, in which only two or three *H-2* alleles were segregating. The fourth location (farm KE) was a big barn with a granary and a corncrib which could have been occupied by two demes. This would explain the higher number of segregating *H-2* alleles (seven different H-2 phenotypes were found on this location).

The present data also support the classification of H-2 antigens into two groups: "private" antigens which are limited to only a few *H-2* alleles, and "public" antigens which are shared by most of the *H-2* alleles. Thus, for instance, in the laboratory strains the antigen H-2.4 is limited to allele $H\text{-}2^d$ and its recombinant derivatives ($H\text{-}2^a$, $H\text{-}2^i$, $H\text{-}2^t$, and $H\text{-}2^y$). In agreement

Table 1. Antiserums used for the typing of wild mice.

Code designation	Antiserum made in		Immunizing tissue supplied by		Antibodies present
	Strain	*H-2* allele	Strain	*H-2* allele	
C-1*	(C3H.SW × B10.M)F_1	b × f	C3H.NB	p	1, 16
S-30	(DBA/1 × DBA/2)F_1	q × d	CR	r	1, 18
C-2*	(B10.D2 × A)F_1	d × a	B10.A(2R)	h	2
C-3*	(A.BY × WB/Re)F_1	b × w	A.SW	s	1, 3, 19
120	(B10 × A.CA)F_1	b × f	A.SW	s	3, 19
C-4*	(C3H.SW × AKR.M)F_1	b × m	B10.A	a	4
S-29	(A.BY × AKR.M)F_1	b × m	A	a	4
C-5*	(B10.D2 × A.CA)F_1	d × f	B10.A(5R)	i	5, 33
S-31	(B10.D2 × HTG)F_1	d × g	B10	b	5, 33
C-7*	(C3H.SW × B10.A)F_1	b × a	C3H.NB	p	7, 16
135	(B10 × A.SW)F_1	b × s	A.CA	f	8, 9
C-9*	(B10.D2 × A.SW)F_1	d × s	B10.M	f	9
S-22	(B10.A × A.SW)F_1	a × s	A.CA	f	9
C-11*	(B10.D2 × C3H.NB)F_1	d × p	LP.RIII	r	11, 25
S-16	(C3H.DT × BF)F_1	d × n	C3H.R3	r	11, 18, 25
C-16*	(B10.A × A.CA)F_1	a × f	B10.Y	pa	16
S-21	A	a	C3H.Q	q	17, 30
S-24	(B10 × A)F_1	b × a	A.SW	s	7, 19
C-22*	(B10.A(2R) × DBA/1)F_1	h × q	B10	b	22
C-23*	(B10 × LP.RIII)F_1	b × r	B10.BR	k	23, 32
S-32	(B10 × CR)F_1	b × r	B10.K	k	23, 32
S-2	(B10 × AQR)F_1	b × y	DBA/1	q	30
S-33	(B10.A × AKR)F_1	a × k	AKR.M	m	30

* Antiserums supplied by Transplantation Immunology Branch, National Institutes of Health, Bethesda, Maryland.

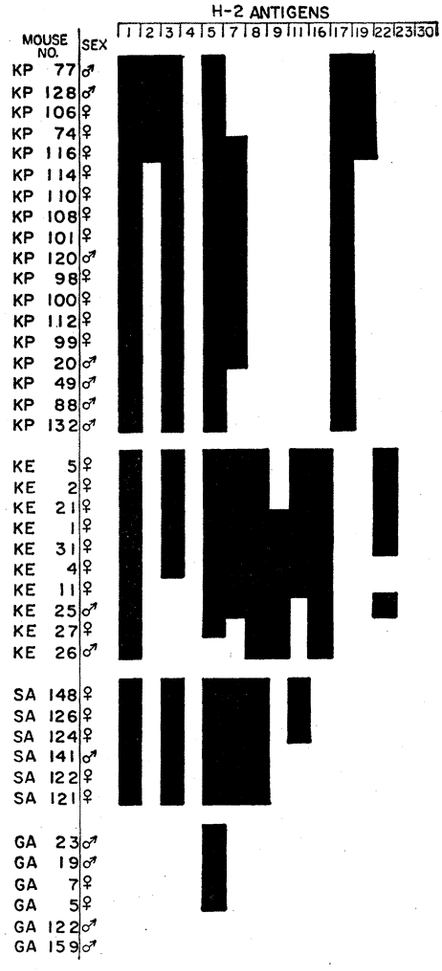

Fig. 1. Distribution of 15 antigens of the H-2 system among 40 wild mice. Each solid line represents the presence of an antigen. Absence of an antigen is represented by an empty space. Mice from the same location have the same capital letters.

with this, antigen H-2.4 was not found in a single wild animal despite the fact that it is one of the strongest H-2 antigens and could hardly be missed in typing. The same holds for antigens H-2.23 and H-2.30 which were also absent in our sample of wild mice. Antigens H-2.2, 9, 16, 17, 19, and 22, which according to the H-2 chart can also be considered as private, were found in the wild mice, but their occurrence was limited to only one farm. On the other hand, antigens H-2.1, 3, 5, 7, and 8, which are widely distributed in the H-2 chart of laboratory mice and are therefore considered public, were also widely distributed in the wild population.

The strength of reaction of red blood cells from wild mice with antiserums against antigens H-2.1, 3, 5, and 8 was comparable with that of red blood cells of the positive inbred strains. The reactions of antiserums against antigens H-2.11, 16, 17, 19, and 22 were usually weaker with red blood cells of wild mice than with red blood cells of the positive inbred strains. To what extent cross-reactivity is responsible for this difference we have no way of knowing.

The cells of some wild mice did not react with any of the 23 antiserums and some others reacted only with one antiserum (mice from farm GA). This could be due either to reduced expression of the H-2 antigens on red blood cells of these mice or to the absence of known H-2 antigens and their replacement by others as yet undetected.

All 40 wild mice were also typed with a rabbit antimouse serum for the presence of the serum protein substance (Ss) controlled by the H-2 region (*10*). The immunodiffusion tests revealed a high level of Ss in all mice. The Ss^h/Ss^h genotype of all males and two females was confirmed by progeny tests. The rest of the females, from whom no progeny were obtained, could have been either of Ss^h/Ss^h or of Ss^h/Ss^1 genotype, since the heterozygous phenotype cannot be reliably distinguished from the high homozygous phenotype on an undefined genetic background.

The direct hemagglutination typing of wild mice with antiserums produced in laboratory animals has, of course, its drawbacks, most obvious of which are the following:

1) A negative result does not necessarily mean an absence of an antigen, since such phenomena as dosage effect and differential tissue distribution play an important role in the typing.

2) The combination of antigens found in an individual animal does not necessarily represent an *H-2* allele, since the animal could be a heterozygote.

3) A positive reaction of a monospecific antiserum does not mean that the detected antigen must be identical with the one against whom the antiserum was prepared, since cross-reactivity with a related antigen cannot be excluded.

4) Antiserums produced against laboratory mice and then applied to the study of wild mice can probably detect only a small part of the antigenic polymorphism of the wild animals, since there are probably many other presently undetected antigens in the wild population.

However, at least some of these drawbacks can be counterbalanced by addition of absorption analysis, progeny studies, genetic analysis, and cross-immunizations of the wild mice. Experiments along this line are already in progress. We hope that the results of such combined studies will allow us to estimate rather precisely the extent of the H-2 polymorphism. Since the *H-2* system is such a close homolog of the *HL-A* system in man, this might have some important bearing on the perspectives of clinical transplantation. However, even with this limited knowledge about the H-2 antigens in wild mice, it is obvious that antigens can be extremely useful in such areas as the study of population dynamics and ecology of this species.

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Indian Muntjac, Muntiacus muntjak: A Deer with a Low Diploid Chromosome Number

Abstract. *The Indian muntjac* (Muntiacus muntjak) *has a diploid chromosome number of 7 in the male and 6 in the female, the lowest number yet described in a mammal. Its near relative, Reeve's muntjac* (Muntiacus reevesi) *has a diploid number of 46, and the karyotypes of the two species are very different.*

The deer family, Cervidae, probably arose from traguloid ancestors in the Oligocene. The main line evolved in Eurasia, eventually spread to the New World and culminated in the two largest cervid subfamilies, the Odocoileinae and the Cervinae (*1*). These subfamilies contain 34 species (*2*). There are two primitive cervid subfamilies, the Muntiacinae (muntjacs and tufted deer) and the Moschinae, both of which are solely Asian. The former is represented by six species, and the latter by a single species, the musk deer, *Moschus moschiferus*. The Muntiacinae and Moschinae are considered to be representatives of an early branch from the main cervid line that has evolved with little or no divergence (*3*).

Mammalian chromosome numbers vary from 10 in the female, and 11 in the male of the marsupial *Protemnodon bicolor* (*4*) to 84 in the black rhinoceros, *Diceros bicornis* (*5*). Thirteen species of the Odocoileinae and Cervinae have diploid numbers of 56 to 70 but a relatively constant fundamental number (FN) of 70 to 74, that is, number of major chromosomal arms. Of the two primitive deer subfamilies the Reeve's muntjac, *Muntiacus reevesi*, has a diploid number of 46 (FN of 46), and its near relative, the Indian muntjac, *M. muntjak*, was reported to have a diploid number of 6 (*6*). We now confirm, after the study of two male and one female muntjacs, that this species has this unusually low diploid number of 6 for the female and 7 for the male (Fig. 1).