RESEARCH ARTICLE

Evolution in Inbred Strains of Mice Appears Rapid

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Abstract. *Genetic variation at 97 loci in ten commonly used inbred strains of mice is greatly in excess of that expected under current assumptions. Evidence against all of the readily apparent explanations is presented and the possibility of early selection for heterozygosity or of conversion is suggested. The common ancestor of these strains is estimated to have occurred about 150 years ago.*

Groups of organisms for which large amounts of genetic data are available and whose evolutionary history is accurately known are important for testing hypotheses about general evolutionary mechanisms. Unfortunately, such groups of taxa are rare. One is the house mouse, *Mus domesticus* and its inbred strains. Over 700 genetic loci have been described from about 250 distinct inbred lines whose evolutionary history is often well known. Linkage group affinities are known for more than 400 of these loci. Extensive biochemical differences at individual loci exist between strains and substrains so that genetic divergence can be measured unequivocally (*1*).

Inbred strains are produced by several mating systems although the most widespread method is inbreeding between full-sibs. An inbred strain is operationally defined as all the descendants of a single brother–sister pair of mice produced by 20 or more generations of full-sib inbreeding so that the probability of heterozygosity at any unselected locus is less than 0.02 (*2*).

While evaluating different methods of reconstructing the phylogeny of the inbred strains of mice, we discovered unexpectedly large amounts of genetic divergence among strains. We now describe this divergence in ten commonly studied strains and explore different hypotheses that would explain it.

The amount of genetic divergence among inbred strains is a function of four factors: (i) initial heterozygosity in the common ancestral population, (ii) residual heterozygosity after n generations of inbreeding, (iii) contamination from outcrossing, and (iv) mutation. These factors are not necessarily independent; however, their relative contribution is important in determining the appropriateness of inbred strains for studies of evolutionary mechanisms. After inbreeding to homozygosity, the genotype of an inbred strain (in the absence of selection) represents a random gamete from the ancestral population. Therefore, two different inbred strains should have differences equal to h times the number of loci examined where h is the heterozygosity (diversity) of these loci in their ancestral population.

Contamination arising from human error is often suspected as the primary cause of genetic heterogeneity when the variability encountered significantly exceeds that expected from the other three factors. Unfortunately, unless several discriminating marker genes are involved simultaneously, contamination is difficult to distinguish from mutation. When contamination is detected or suspected by commercial breeders, the contaminated mouse stock is usually destroyed.

The relative magnitude of contamination in stocks maintained by commercial animal breeders is unknown. Indirect evidence from genetic analyses of divergence among genetic strains has been used to suggest that contamination has occurred (*3, 4*). However, Roderick (*5*) examined sublines of a number of strains from the Jackson Laboratory over a 10-year period and found no evidence of genetic heterogeneity within strains for 13 loci. Similar findings have been reported (*6, 7*). It is reasonable to assume that contamination may have been a more significant factor earlier in the development of inbred lines when quality control procedures were less refined. Incomplete inbreeding or a single generation of outcrossing could significantly retard the effects of many generations of inbreeding between full-sibs (*8*).

Strains of mice. The ten strains of mice examined (together with their coat color) were A/HeJ (albino), AKR/J (albino), BALB/cJ (albino), CBA/J (agouti), C3H/HeJ (agouti), C57BL/6J (black), C57BR/cdJ (brown), C58/J (black), DBA/1J (gray), and DBA/2J (gray). Coat colors are given to assess the probability of interstrain contamination. The known genetical relations among these inbred strains (Fig. 1) were described by Staats (*9*) and Festing (*10*).

Loci examined. Staats (*9*) gives inbred strain distributions for 158 loci. Loci were included in this analysis if data were available for at least eight of the ten strains examined here. This resulted in 97 loci being included in these analyses, considerably more than have been examined in previous studies of divergence among inbred strains [for example (*11*)] or wild populations.

All data were for Jackson Laboratory strains except that, where data on Jackson strains were missing, other substrain data were used provided that all substrains possessed the same allele at that locus. Twenty-three loci are unvaried among these ten strains, eight loci contain only unique variants (occurring only in one strain), and 66 loci are cladistically informative (at least two nonunique alleles) (*12*). Of the 97 loci, 62 encode proteins, 33 are related to immune functions, and two are unassigned. All 97 loci were used in all parts of our study. The number of alleles at these loci in these strains averages 2.01, with three loci (*Hba, H-2, Igh-1*) having five alleles, three loci (*Aox-1, Igh-2, Mls*) having four alleles, nine loci having three alleles, 59 loci having only two alleles, and the remaining 23 having only one allele. In 12 other strains not discussed here, three of these loci (those already showing five alleles) possess two or more alleles not present here, while eight other loci possess one extra allele. One of the latter loci, *Igh-2*, already has four alleles in this study. Eleven of the 15 loci with more than two alleles are related to the immune system. All of the 87 loci whose chromosomal location is known are autosomal.

Data from the cladistically informative loci are more than 96 percent complete with only 23 of 660 possible data points missing. Data for 47 of the 66 loci are

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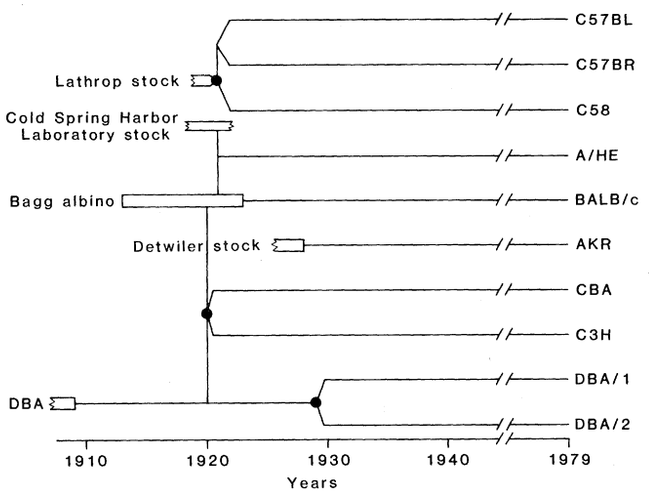

Fig. 1. Phylogenetic relations of the ten strains of mice examined in this study. The thin lines show periods of brother × sister mating, the box-like lines show periods where random breeding is known (boxes have smooth ends) or is assumed (boxes have jagged ends) to have occurred. Nodes with a solid circle descend to taxa used to construct Fig. 3.

complete. One strain (C58) is missing seven data points, three strains are missing four, and the rest are missing two or less. AKR, C57BL, and DBA/2 have complete data.

For the cladistically noninformative loci, 23 loci are homoallelic, seven have two alleles one of which is unique to one strain, and one locus (*Lv*, δ aminolevulinate dehydratase) has three alleles, two of which are each unique to its strain. These 31 loci are similar to the other 66 loci except for having more missing data (10 percent) and containing a larger fraction of loci coding for proteins.

Estimating heterozygosity and divergence. Divergence between two inbred strains may arise through any of four factors: (i) different alleles may have been fixed in the two inbred lines at loci that were polymorphic in the ancestral population, (ii) some polymorphic loci may not yet have gone to fixation in one or the other of the inbred lines (residual heterozygosity), (iii) new alleles created by mutation may have been fixed, and (iv) new alleles, introduced by contamination from another line, may have been fixed.

The equation for divergence describing the fraction of loci that is different is:

$$d_{ij} = c_{ij}h + 2my_{ij}(1 - c_{ij}h) \quad (1)$$

where d_{ij} is the expected fraction of allelic differences per locus between inbred strains i and j over the loci examined, h is the heterozygosity at these loci in the ancestral wild population, c_{ij} is the correction factor for h as a result of any genetic relationship between i and j [(1 − the coefficient of kinship) where c_{ij} is 0.75 for full-sibs], m is the rate of fixation of alleles per year in these strains, and y_{ij} is the number of years since strains i and j were separated. For this equation, c_{ij} is known and y_{ij} can be reasonably estimated.

The first term on the right of Eq. 1 is the divergence arising from the reduction of polymorphism present in the population at the time the strains were separated (initial plus residual heterozygosity). The second term is the divergence accumulated by the fixation of newly arising or introduced variants since the time of strain separation. The term in parentheses corrects for the inability to observe new differences in positions that were already different at the time the two lineages separated.

Genetic relationships among these ten strains give the values of c_{ij}. For these strains, a value of $c_{ij} = 1.0$ is probably valid only for the 21 comparisons of C57BL, C57BR, and C58 versus the other seven lineages.

As C57BL and C57BR were derived from a cross of a single Lathrop stock male and female in 1921, their gametes are related as full-sibs ($c_{ij} = 0.75$) and 25 percent of the alleles are identical by descent. The C58 strain arose from the same male as C57 but a different female; C58 is related to the two C57 strains as a half-sib ($c_{ij} = 0.875$) and 12.5 percent of the parentally derived alleles are identical by descent.

Strains CBA and C3H both arose in 1920 from a single cross of the Bagg and DBA lines. The DBA line had been inbred since 1909 and the Bagg line would later be inbred to produce BALB/c. Therefore, CBA and C3H should be identical by descent for all alleles arising from the DBA line and related as full-sibs for those alleles from the Bagg line ($c_{ij} = 0.375$). Strains DBA/1 and DBA/2 were created in 1929 by separation of the already inbred DBA stock; thus, all their alleles should be identical by descent ($c_{ij} = 0$), assuming that inbreeding was complete after the 20 years of inbreeding.

Of the 45 pairwise comparisons described here, the numbers of pairwise comparisons involving $c_{ij} = 0.875$, 0.75, 0.375, and 0 are 2, 1, 1, and 1, respectively.

Phylogenetic relationships among strains. A phylogeny obtained by parsimony analysis (*13*) of these ten strains for the 97 loci is given in Fig. 2. Other phylogenetic methods, including UPGMA (*14*), EVOLVES (*15*), neighborliness (*16*), and distance Wagner (*17*) all give the same topology (but not necessarily the same branch lengths) except for the group containing C57 and C58, where no method should be expected to separate C58 from the C57 strains since the former's ancestors were half-sibs of the latter's ancestors which were full-sibs. The AKR strain, whose genealogy is not well known, is shown to be related to the DBA, CBA, and C3H strains.

Since in all these methods a bifurcating genealogy is assumed, the CBA and C3H strains are made the sister group of one of the hybridizing lines. Moreover, the methods all choose the inbred DBA lineage as the sister group and this should be expected since CBA and C3H should share more alleles with descendants of their inbred parent than with descendants of their outbred parent.

This level of congruence in results obtained by means of different phylogenetic algorithms is unusual since analyses of other biological data by different methods often give quite divergent results. This faithful reproduction of the known genealogy of the strains and the congruence of the results among different methods argue strongly for the robustness of these data to reproduce the actual phylogenetic history of these ten strains.

A total of 169 gene substitutions is required to explain the divergence but substitutions in the earlier branches are more properly interpreted as changing originally polymorphic loci to distinct monomorphic loci.

The amount of divergence between strains. There are extensive differences among these ten inbred strains of mice for the 97 loci. Percentage difference ranges from 14 percent between DBA/1 and DBA/2 to 54 percent between DBA/2 and C57BL (Table 1). The mean divergence over all strains and loci (and standard deviation) is 0.40 (±0.10) while that between the group containing C57 and C58 and all others is 0.47 (±0.04).

Equation 1 can be rearranged to give

$$d_{ij} = c_{ij}h(1 - 2my_{ij}) + 2my_{ij} \quad (2)$$

The estimates of divergence, d_{ij}, for the five pairs of mouse strains having $c_{ij} < 1.0$ are shown in Fig. 3. The d_{ij}

values for the line in Fig. 3 were adjusted to a divergence time of 58 years, a value determined by assuming all the genetic data were, on average, described in the year 1979, the date of the Festing reference (*10*). The divergence time for C57BL, C57BR, and C58 (which arose in 1921) is 58 years while that for the C3H and CBA strains (which arose in 1920), is 59 years. The divergence value in Table 1 of the C3H and CBA was standardized to a time scale of 58 years by multiplying 0.20 by 58/59. The two DBA strains originated in 1929 and hence had only 50 years of divergence. Accordingly, their divergence in Table 1 was multiplied by 58/50.

The least-squares regression equation (with standard errors for slope and intercept) of divergence onto 1 − the coefficient of kinship is

$$d_{ij} = 0.165(\pm 0.029) + 0.079(\pm 0.043)(c_{ij})$$

The intercept is significant with lower and upper 95 percent confidence limits of 0.072 and 0.257, respectively. The regression slope is not significantly different from zero with a probability associated with $b \neq 0$ of between 0.1 and 0.2. The intercept of the line, determined largely by the DBA/1 and DBA/2 comparison, is the divergence after 58 years, and provides a fixation rate of $0.165/116 = 1.4 \times 10^{-3}$ fixations per locus per year. Substituting the upper and lower 95 percent confidence limits values for the intercept, we obtain fixation rates ranging from 6.2×10^{-4} to 2.2×10^{-3} fixations per locus per year.

Heterozygosity per locus. Our estimate of the heterozygosity for the ancestral mouse population from which these stocks arose [obtained by dividing the slope of the line in Fig. 3 by (1 − the intercept)] equals 0.09. The relatively large standard error of the slope makes the estimate of the original heterozygosity uncertain. However, this value is the same as the 0.09 estimated for four populations of *Mus musculus musculus* (*18*). Estimates of original heterozygosity for wild populations of *M. m. domesticus* (*18, 19*) range from 0.06 to 0.09, while the estimate for *M. m. brevirostris* is 0.11 (*18*). If a new population were created by mixing equal numbers of each of these ten strains, its heterozygosity would be 0.33.

Approximate age of ancestral populations. It is not known when the ancestors of these inbred lines were derived from wild populations. The original stock is assumed to have been derived from the pet mouse trade, possibly from a single region in England (*4*). Based on studies of mitochondrial DNA (*20*), it has been suggested that the "old" inbred strains of mice (including those strains examined here) were derived from a single female of *Mus domesticus*, which is the house mouse of Western Europe and the Mediterranean region. The mitochondrial DNA type found in these old strains occurs in only 4 percent of wild *M. domesticus* surveyed.

We do not know when the Bagg, Lathrop, DBA, Detwiler, and Cold Spring Harbor stocks that gave rise to these inbred lines were separated from each other nor do we know their level of inbreeding. They probably did not all become separated at the same time. Nevertheless, an average time of separation can be estimated from the block of 7 × 3 divergence values in the upper right half of Table 1 which represent the group containing C57 and C58 against the others. This average divergence is 0.47 ($\sigma = 0.042$), which is five times the original heterozygosity. Substituting into Eq. 1, we obtain

$$0.47 = 0.09 + 2y(1.4 \times 10^{-3})(1 - 0.09)$$

Thus, y, the time of divergence of these strains, is approximately 150 years prior to Festing (*10*) or about 1830. This is a reasonable estimate in view of the history of mouse stocks and the mitochondrial DNA evidence.

Other results. None of the results reported are materially changed by examining the protein loci separately from the immunological loci except that the overall rate of divergence is greater for the immunological loci and the confidence intervals are greater for each. We are unable to detect any nonrandomness (with respect to the chromosomes on which the divergence occurred) between pairs of strains when corrected for the number of loci and alleles.

Discussion of divergence and heterozygosity. The divergence seen among these strains may have its source among any of four factors, not including systematic biases which will be discussed presently. The first of these factors is initial heterozygosity. Our value of 0.09 has a high standard deviation (0.04) but it is consistent with estimates from other mouse populations on both sides of the Atlantic Ocean. Moreover, it is six standard deviations below the mean heterozygosity of a pool of these ten strains. Hence, it is difficult to accept the proposition that the observed divergence is the simple consequence of the segregation of original heterozygosity.

The second factor is residual heterozygosity. Since these strains appear to be fixed for one allele at each of these loci in each of these strains (as they should be after more than 50 years of reported full-sib mating for the youngest of these strains), residual heterozygosity cannot be a significant source of observed divergence. This leads to consideration of the fixation rate of new variants and the source of these new variants.

Fixation rates. The value of 1.4×10^{-3} for fixation rate is very high. On the other hand, since these are inbred lines produced by brother × sister matings, the effective population size is only two; hence, a rapid fixation rate is reasonable provided there is a store of genetic diversity on which to operate. Since the original supply of genic diversity was estimated to be only 0.09, most of the currently observed diversity would appear to have arisen thereafter.

Rate of introduction of new variants. Since the effective (and true) population size (N) is only two, a newly introduced variant, even if deleterious, has nearly one chance in four of being fixed if it is not a gene being monitored for purity of the lineage. The fixation rate (*21*) is

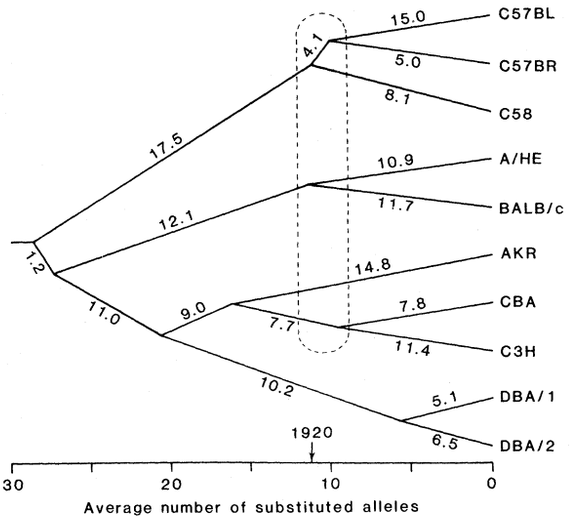

Fig. 2. An example of the phylogeny obtained by parsimony analysis of the 97 loci by the method of Fitch (*13*). Other methods gave the same topology (but not necessarily the same branch lengths) except for the group containing C57 and C58 for which all three pairs are expected to have had eight or nine allelic differences in 97 loci at the time of their separation. A total of 169 gene substitutions are required to explain the divergence, but those in the earlier branches are more properly interpreted as a change from originally polymorphic loci to different monomorphic loci.

$2NuF$, where u is the rate of introduction of new variants, s is the selection coefficient, and F, the probability that a new variant will be fixed, is

$$F = (1 - e^{-2s})/(1 - e^{-4Ns}) \quad (3)$$

For small values of Ns (<0.05), Eq. 3 reduces to $1/2N$ and the fixation rate $= 2Nu/2N = u$, the introduction rate. If contamination (or biased data) could be ruled out as the source of the new variants, then u would be the mutation rate. We therefore estimate by this method that the mutation rate could be as great as to be 1.4×10^{-3} mutations per locus per year or 5×10^{-4} per generation, an estimate several orders of magnitude greater than the conventional estimates in mice (*22*).

Are the strains significantly contaminated? Many of the inbred mouse strains may have been contaminated through outbreeding with other mice and, on the surface, contamination might seem the easiest explanation for the high rate of divergence among strains. While we do not wish to discount the possible presence of contamination, we believe that contamination cannot readily account for our observations for several reasons as follows:

1) Contamination cannot be from mice of these or closely related strains since this would have the effect of reducing differences.

2) There would need to be several different contaminating mice, each one more than 40 percent different from the others, because the major groups derived from the Bagg, DBA, and Lathrop stocks are all different from each other by that amount.

3) Wild *Mus domesticus* are not sufficiently divergent to be the source of divergence. There are data (*23*) on three inbred strains (C57BL/6, BALB/c, and DBA) and eight mice from Morocco, Spain, England, Italy, Switzerland, and Yugoslavia that have been assayed for 56 protein loci. The probability of two gametes, one from each pool, having different alleles at a given locus is only 0.136.

4) The contaminating mice would have had to introduce their divergent genes in a very special way. As described above, we have attempted to determine the phylogenetic relationships of these ten strains using a number of different algorithms including UPGMA (*14*), parsimony (*13*), EVOLVES (*15*), neighborliness (*16*), and distance Wagner (*17*) and, unlike the case of most biological data, all algorithms gave the same tree except for their inability to separate the half-sib C58 from the full-sib C57 strains. Moreover, the tree produced by these methods was identical to the phylogeny as we know it. It is unlikely that contamination could occur in such a widespread fashion and still preserve the phylogenetic information so well that five different methods all correctly obtained the known relationships and completely agreed on those that are not known.

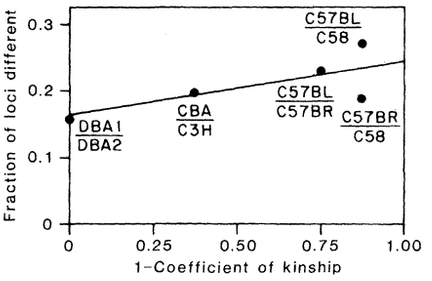

Fig. 3. Plot of d_{ij} as a function of c_{ij} from the equation $d_{ij} = c_{ij}h(1 - 2my_{ij}) + 2my_{ij}$ with $y_{ij} = 58$ years. The linear regression gives $d_{ij} = 0.079$ $(\pm 0.043)(c_{ij}) + 0.165$ (± 0.029). Distances are from Table 1 adjusted to 58 years (age of the C57 and C58 separation).

5) We have developed a method to detect hybridization and it clearly identifies the group containing CBA and C3H as a hybrid of the DBA/1–DBA/2 group and the A–BALB/c group. It is difficult to imagine the preservation of the hybrid information in the face of great contamination of the strains.

It would be possible to discount both reasons 4 and 5 if all the contamination occurred prior to all known strain separation. However, this would prevent contamination from being the explanation for the divergence occurring after the separation within the Bagg and DBA strains.

6) It is possible to consider the root of the phylogenetic tree (Fig. 2) to be in 1830 and the tips in 1979 on the basis of the analysis from Fig. 3 (which does not involve parsimony). In that case, 1920 would fall at the point shown by the arrow (Fig. 2) if time were proportional to distance. The three strain separations that occurred in 1920 and 1921 are those nodes within the dashed lines on the tree where distance is proportional to the number of allelic substitutions during descent. The correspondence of the two suggests that the whole process is behaving in a clock-like manner. Such behavior would not seem to be a likely result in the presence of serious, multiple contamination problems.

One might also note that contamination would need to be explained by male interlopers since mitochondrial DNA restriction analysis has indicated that all these strains arose from a single ancestral female (*20*).

Biases in the data. Divergence among strains might be explained by several potential systematic biases in the data. There are at least eight as follows:

1) Loci that do not vary may go unreported. Unvarying loci may not be reported because of their apparent inutility. The extent to which this might occur is unknown but, if it exists, it would cause our estimate of divergence to be too high. However, it does not seem likely that there are 300 unreported, unvaried loci, the number required to reduce the average divergence from 0.40 to 0.10.

2) Breeders may consciously or unconsciously select for mice that are healthy, docile, have large litters, mature early, or have long reproductive periods. However, it does not appear that this practice would have any systematic effect on which alleles would be fixed. We will, however, propose a way in which it might affect the estimated initial heterozygosity.

3) Missing data may affect the calculations. The effect would depend upon whether those loci that have missing data are more or less divergent than those loci for which there are complete data. Since there is no reason to believe loci that are missing data are different from those that are not, there is no a priori directional bias. Moreover, since the amount of missing data is small, its effects could not alter greatly the total amount of divergence.

4) Inbreeding may have occurred in the stocks prior to brother × sister mating. Previous inbreeding would cause the allelic difference at the time of instituting sibling mating in the group containing C57 and C58 and in the group containing CBA and C3H to be overestimated. This, in turn, would lead to an underestimation of the amount of divergence since inbreeding began and, as a result, give an estimation of the rate of fixation that is too low.

5) Loci monitored as diagnostic for a strain will not be permitted to change. Hence, the estimated rate of fixation would be too low.

6) The analytical methods may have failed to detect all the genic diversity present. This would cause the estimation of the amount and, therefore, the rate of fixation to be too low.

7) We do not know exactly when the alleles at various loci in a strain were determined. The majority of genotypes were obtained from Festing (*10*) so that, to the extent that average date of fixation is prior to 1979, the estimation of the fixation rate is too low.

8) Equation 3 used to calculate the

divergence rate errs to the extent that it assumes that all deleterious variants have an s value between 0 and 0.025. For larger values of s, the fixation rate must underestimate the rate at which new variants are introduced.

Only bias 1 would exaggerate the rate of divergence and, thus, the fixation rate. However, it does seem unlikely that there are 300 examined but unreported invariant loci, which is the number required to make the estimated fixation rate of 1.4×10^{-3} comparable to previous estimates of the mutation rate. Because the evidence appears to be against both bias and contamination explaining these data, the fixation rate may be equal to the mutation rate and, thus, there may be a high mutation rate in these strains (but see below).

Other inferences of a high mutation rate. We are hardly the first to suggest an elevated mutation rate in inbred mouse strains. Evidence for rapid divergence in inbred mouse strains is found in polygenic systems as well. Morphological divergence between sublines of C57BL/GR in the shape of the mandible and various minor skeletal variants strongly suggests rapid divergence by mutation and that subline divergence is a continual phenomenon (*6, 24–26*). Festing (*26*) suggested a linear relationship between divergence in shape of the mandible and pedigree relationship; that is, the longer the time of separation between sublines, the greater the divergence in the shape of the mandible. Hoi-Sen (*25*) suggested that subline differentiation for polygenic traits occurred at a rate much higher than the spontaneous mutation rate usually described for genes with major effects.

Gruneberg (*27*) found a high rate of skeletal change in C57BL sublines and suggested a viral cause. Beardmore (*28*) responded with an alternative interpretation of the high rates of change based, among other things, on the unknown number of genes contributing to a single trait. We believe that the many previous reports of a high mutation rate may have been discounted by virtue of uncertainty in the number of loci involved in polygenic traits. Since our data are for single qualitative loci, they are not subject to this uncertainty and even permit one to suggest that the previous workers' hypothesis of a high mutation rate may have credence after all.

Evidence against a high mutation rate. Johnson *et al.* (*29*) have been studying mutagenesis in crosses between DBA/2J and C57BL/6J. They found no new mutants upon examining 20 loci (all among the 97 we examined) in 3848 progeny of such crosses (76,960 observations) where no mutagen was employed. Thus, their mutation rate is two orders of magnitude below our fixation rate. This is of particular importance as this analysis was on inbred strains of mice.

Table 1. Minimum pairwise differences for 97 loci between ten inbred mouse strains. Values below the diagonal are actual differences and those above the diagonal reflect the proportion of differences for those loci known for both members of the pair.

	C57BL	C57BR	C58	BALB/c	A	AKR	CBA	C3H	DBA/1	DBA/2
C57BL	–	.23	.27	.41	.41	.51	.51	.51	.52	.54
C57BR	20	–	.19	.43	.44	.41	.44	.49	.49	.50
C58	23	15	–	.47	.48	.40	.44	.46	.48	.50
BALB/c	38	36	39	–	.24	.50	.46	.41	.37	.45
A	39	37	40	22	–	.43	.44	.30	.38	.46
AKR	48	35	34	46	40	–	.31	.34	.35	.39
CBA	47	36	36	41	40	28	–	.20	.35	.31
C3H	47	41	38	37	28	31	18	–	.38	.38
DBA/1	49	41	41	34	35	33	32	35	–	.14
DBA/2	43	36	37	36	37	31	24	30	11	–

Other puzzles. In addition to the high fixation rate, our data present two other puzzles.

The second puzzle is that there are few published reports of new mutants (other than those for which new strains are selected) arising within these strains. Surely if mutations are occurring at a rate greater than 10^{-3} per locus per generation, many new mutations should have been observed in the generation of their initial occurrence. The major opportunity to see such new mutations would appear to be within the laboratories of commercial breeders. Absence of such reports may mean they were not looked for, not seen, discounted, or not deemed of sufficient importance to report. This puzzle is not addressed further.

The third puzzle is, why do we see only two different alleles at so many loci? Of the 145 substitutions of one nonunique allele for another, 71 are parallel (or back) substitutions of some allele from among the other 74. At 52 of the 66 cladistically informative loci, there are only two alleles but these 52 loci require 113 of the 169 total substitutions observed and only 41 of these 113 separate the three major ancestral stocks. How can there be so many (at least $113 - 52 = 61$) new mutations being fixed in 52 positions without additional new variants arising? Why are there not three or more known variants at many of these loci rather than only two? The occurrence across these strains of only two variants at the vast majority of the loci might suggest the retention of original heterozygosity from the ancestral population were it not for the fivefold increase in heterozygosity and the clear requirement for the fixation of many new mutants. Another view of this puzzle is that, with an average of only two alleles per locus, the maximum heterozygosity is only 0.5, achieved when both alleles have the same frequency. The group containing C57 and C58 has an average divergence from the other strains of 0.47, which is not significantly different from 0.5. This result implies they are nearly maximally diverged from the others given the allelic composition of these ten strains. Contamination does not readily explain this property of the data.

Reconciliation: Two alternative hypotheses. We have presented evidence against the readily conceivable explanations of these data. The importance of inbred mice and the degree of divergence is so great as to demand that an explanation be found. We propose two hypotheses that, if true, might resolve two of the puzzles described.

The first hypothesis was developed from a suggestion of Dr. J. F. Crow (*30*) that there is direct selection for heterozygosity in the creation of the inbred lines which, through linkage, even affects loci that do not themselves have recessive deleterious alleles. When developing the A strain, Strong (*31*) deliberately chose the most vigorous male and female of each litter to create the next generation because he was concerned that inbreeding depression would wipe out the lineage before it became homozygous at all loci. This regimen almost certainly selected the most heterozygous animals for further breeding. However, fearing that this regimen might still not be enough to ensure survival of the lineage, Strong started several such lines in hopes that at least one lineage might survive the inbreeding crisis.

How heterozygous were the first pair of siblings? Any two randomly selected siblings would be expected to carry three-quarters of the heterozygosity of their parents, but the two most heterozygous mice in the litter might contain a larger fraction. If we assume that each

parent had the population average for heterozygosity of 0.09, then the total fraction of heterozygous loci in their combined pool could be as large as 0.18. This is still considerably below the value that would be needed to explain the many divergences in excess of 0.4. If the procedure of choosing the most vigorous offspring should prove wanting, crossing two such parallel inbred lines might restore vigor, albeit at the cost of slowing the progress to total homozygosity. The effect, however, would again be to expand the effective fraction of loci that were initially heterozygous.

This hypothesis asserts, then, that the divergence we see today was determined very early, largely as the result of initial selection for heterozygosity. If there were only two major alleles at most of these loci originally, this hypothesis would also explain why there are only two alleles present at so many of the loci examined. It also avoids the need for a high mutation rate to explain the diversity. A major difficulty with the hypothesis is that it is not clear how many of the original strains originated in this manner nor how the hypothesis can be tested. Moreover, it cannot account for the divergence between DBA/1 and DBA/2.

A second hypothesis tries to reconcile a possibly high mutation rate and the observation of only two alleles by requiring a mechanism that restricts variability to only two alternatives. Such a mechanism must either produce an increased rate of mutation in the inbred strains specifically or produce it in all strains in a way that is obscured in outbred strains. We know of no evidence that the mutation rate is increased in inbred mouse strains but that is not evidence against it. Indeed, consistent with this idea, Murphy (*32*) described a high frequency of tumors in 16 major inbred mouse stocks, including nine of the ten strains used in this study. In the extreme case, 100 percent of C3H virgin females developed mammary gland tumors in their first 8.8 months. This might arise from a general inbreeding depression but it would also be expected if inbreeding caused an increased mutation rate. Also, balanced recessive lethals can lead to increased mutation rates in regions outside of the chromosomal inversion including other chromosomes (*33*). We do not know of any balanced lethals in these strains but there are known mechanisms that increase mutation rates and the high incidence of tumors supports the suggestion of a high rate of mutation in these strains. This, however, does not explain why we see only two genotypes in 80 percent of the variable loci.

One way around this difficulty is to propose that variants arise from a conversion process in which there are usually only two alternative sources for the converting sequence. Such a process exists for mating type in yeast (*31*). It is not necessary that the converting sequences be complete copies of the locus nor must there be converting sequences for every locus. A mechanism that alternately switched on and off one of two duplicated, but slightly different, loci could also generate just two variants as readily as conversion. Such a mechanism generates phase variation in *Salmonella* in which switching rates as high as 10^{-3} have been observed (*34, 35*).

This mechanism would explain more than the observation of only two different alleles. If this process were occurring in outbred strains, the loci involved would almost certainly appear to be polymorphic and new mutants would be undetected as estimation of the mutation rate in populations normally involves only novel phenotypes not already present. One would need to examine inbred strains specifically to observe this high rate.

That some mutation process, such as conversion or switching, may be occurring at rates as high as 10^{-3} in inbred strains of mice is a testable hypothesis. It may be occurring at high rates in other inbred strains of organisms and even in non-inbred strains, but the means of its detection in the latter are unclear. In view of the low mutation rates observed in a study of inbred mice (*29*), this is probably the weaker of the two hypotheses.

Evolutionary significance. If the extensive divergence is simply the result of the special process required to establish these strains, as hypothesis 1 suggests, there is little of evolutionary significance. If, however, there is a high rate of a special conversion process going on, there are several interesting aspects.

A bottleneck effect, where population sizes are greatly reduced, decreases genetic variance and thereby lessens the organism's ability to respond to new environmental changes. A higher mutation rate would tend to reduce this handicap. Moreover, if the process involves conversion or a switching mechanism and selection has operated to choose alternatives of previous adaptative value, the heterogeneity reintroduced into the active loci might be more advantageous than random mutations. Such a process would represent a new mechanism for providing adaptive versatility to living organisms.

A surprisingly high rate of parallelism in the evolution of proteins was observed early (*36*) and many times since. This would be less surprising if a conversion or switch-like process were operating, although a very limited number of functionally acceptable alternative amino acids might also explain the observation.

Irrespective of the mechanism, if high levels of homozygosity are associated with a high mutation rate, then speciation might sometimes be promoted in small isolated populations by the presence of many mutants. This would give the appearance of a more rapid rate of change at the inception of speciation irrespective of selective advantages of the mutants. More extensive variation could be produced, tested, and integrated than usually occurs. This would also have significance for some aspects of the macroevolution versus microevolution controversy.

Conclusion. The data document a diversity among inbred strains of mice much too great to be accounted for by simple genetic mechanisms. Moreover, we present evidence against the commonly expressed belief that this diversity is the result of contamination of the stocks. We present two alternative mechanisms, conversion and selection for heterozygosity, that could explain the observed divergence. But we are less concerned that one of the alternatives be proved correct than we are that an explanation for the documented divergence be sought that does not rely on the too facile explanation of contamination.

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Es-10 (esterases); *Fv-1*, *Fv-2* (Friend leukemia virus susceptibility); *Gdc-1* (L-glycerol 3-phosphate dehydrogenase); *Glk* (galactokinase); *Gpd-1* (glucose-6-phosphate dehydrogenase-1); *Gpi-1* (glucose phosphate isomerase); *Gus-r*, *Gus-s* (β-glucuronidase); *Gv-1* (Gross virus antigen-1); *H-1*, *H-2*, *H-7*, *H-13* (histocompatibility complex); *Hba*, *Hbb* (hemoglobins α and β); *Hc* (hemolytic complement); *Idh-1* (isocitrate dehydrogenase-1); *If-1* (NDV-induced circulating interferon); *Igh-1*, *Igh-2*, *Igh-Src*, *Igk-V* (immunoglobulins); *Lap-1* (leucine arylaminopeptidase-1); *Lvp-1* (major liver protein-1); *Lyt-1*, *Lyt-2*, *Lyt-3*, *Lyt-4* (T-lymphocyte cell alloantigens); *Lyb-2* (B-lymphocyte alloantigen); *Map-1* (mannosidase sialylation); *Mls* (minor mixed lymphocyte-stimulating locus); *Mod-1* (malate enzyme); *Mup-1* (major urinary protein-1); *Pep-3* (peptidase-3); *Pgk-2* (phosphoglycerate kinase-2); *Pgm-1* (phosphoglucomutase-1); *Pre-1*, *Pre-2* (prealbumins); *Qa-2*, *Qa-3* (Qa lymphocyte antigens); *Qed-1* (Qed lymphocyte antigen-1); *rd* (retinal degeneration); *Rnr* (renin regulation); *Sas-1* (serum antigenic substance-1); *Sep-1* (serum protein-1); *Slp* (sex limited protein within H-2S); *Ss* (serum serological, controls variability in a complement component); *Svp-1* (seminal vesicle protein-1); *Tam-1* (tosyl arginine methylesterase-1); *Tla* (thymus leukemia antigen).

The cladistically non-informative loci examined are: *Ags* (α-galactosidase); *Amy-1*, *Amy-2*, (amylases); *Apk* (acid phosphatase-kidney); *Apl* (acid phosphatase-liver); *C-3* (complement component-3); *Car-1* (carbonic anhydrase-1); *Ea-2*, *Ea-4* (erythrocyte antigens); *Eg* (endoplasmic glucuronidase); *Es-2*, *Es-6*, *Es-8*, *Es-13* (esterases); *Gdr-1*, *Gdr-2* (glucose 6-phosphate dehydrogenase regulator); *Gpt-1* (glutamic-pyruvic transaminase-1); *Got-1*, *Got-2* (glutamate oxaloacetate transaminases); *Gr-1* (glutathione reductase-1); *Lv* (δ-aminolevulinate dehydratase); *Ly-5* (lymphocyte antigen-5); *Mph-1* (macrophage antigen-1); *Np-1* (nucleoside phosphorylase); *Pgd* (6-phosphogluconate dehydrogenase); *Pgm-2* (phosphoglucomutase-2); *Raf* (regulation of α-fetoprotein); and *Trf* (transferrin).

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37. Supported by grants from the National Science Foundation DEB-7814197 to W.M.F. and DEB-8109904 to W.R.A. We are deeply indebted to J. F. Crow for his critical comments and helpful suggestions on several versions of this manuscript. We are also indebted to J. Felsenstein, R. Lande, R. Milkman, M. Nei, F. J. Rohlf, J. J. Rutledge, R. Sage, M. Slatkin, R. Siegel, J. Staats, B. A. Taylor, C. Taylor, M. Turelli, M. Wade, B. Walsh, and A. Wilson for their critical comments on various drafts of this manuscript. Contribution number 2789 from the Laboratory of Genetics, University of Wisconsin.

27 April 1984; accepted 13 February 1985

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