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Selective breeding for variations in patterns of mystacial vibrissae of mice

Bilaterally symmetrical strains derived from ICR stock

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ABSTRACT: The establishment of certain patterns of mystacial vibrissae in mice has been the aim of an extensive breeding program carried on in this laboratory since 1977. In a companion paper we have reported on variations in this pattern in an outbred population of ICR mice. Starting with 21 ICR animals we bred, mostly by brother-sister mating, for 13 bilaterally symmetric patterns of mystacial vibrissae characterized by the presence (or absence) of supernumerary whiskers (SWs). The strains are classified as follows: I, a mouse strain with the standard pattern; II, eight strains bred for the occurrence of SWs at a given site or sites; and III, four mouse strains bred for a maximal number of SWs in different regions of the whiskerpad. Commonly, SWs occur in regions that coincide with the zones of mergence between the three facial processes except for two class II strains in which we bred for SWs in the "straddler" row of vibrissae, and for one class III strain, in which we cultivated the tendency (that appeared late in our program) to have SWs at the crest of a facial process. For classes I and II we analyzed the results for about 18 generations in terms of "improvement," meaning an increase in the percentages of animals with the desired phenotype together with a decreased frequency of undesired SWs. For class III, success in breeding meant the increase of the mean number of the desired SWs. All results led to the same conclusion: there is a genetic basis for the occurrence of SWs. The side preference of a particular SW is not strain dependent. It disappears in those class I and II strains in which almost 100% of animals obtained the desired phenotype. The increase in number of SWs in one zone of mergence does not depend on the presence of SWs in the other. Where tested, we almost always found a representation of an SW in a topologically equivalent location within the "barrelfield" area of the somatosensory cerebral cortex. Except for some diseases early in the breeding program, and some side effects of inbreeding that were eliminated, the population was without obvious defects. Where tested, there was no correlation between the occurrence of SWs and sex. The observed variations in pattern of mystacial vibrissae and their genetic background led us to propose a morphogenetic model for the formation of the pattern of mystacial vibrissae.

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ATTEMPTS to breed selectively for countable characters have been reported by many investigators. They include studies on the number of bristles in *Drosophila melanogaster*[19,25,26,30], the number of fin-rays in *Lebistes*[33], and the number of digits in rodents[14,50]. We describe here the results of breeding experiments in mice for variations in patterns of mystacial vibrissae. Vibrissae of mice are part of highly mobile, sensory organs that, on the basis of their localization on the animal's body, have been subdivided into primary vibrissae (mystacial vibrissae) and secondary vibrissae (the ensemble of supra- and post-orbital, post-oral, inter-ramal, and ulnar-carpal vibrissae)[6]. The number of vibrissae in each subdivision is virtually constant. The introduction of the tabby and crinkled genes into populations of mice led to a decrease in the mean number of secondary vibrissae[7–10]. Subsequent selection for the number of secondary vibrissae led to the es-

tablishment of a group with a low number of vibrissae and to one with a standard number. In some lines the number of primary vibrissae also varied from standard; although details are lacking, their mean number was reported to have decreased[10].

Our interest in the mystacial vibrissae of the mouse stems from the fact that the cerebral representation of these sensory organs occupies a large area (the "barrelfield") in the somato-sensory cortex, and is visible in conventional histological sections cut parallel to the pia above this area[48]. Each vibrissal follicle is represented by a "barrel"—an agglomerate of neurons—in layer IV. The arrangement of barrels is homeomorphic with that of the vibrissae. The part of the barrelfield to which the large mystacial vibrissae (whiskers) project was called posteromedial barrel subfield. For these vibrissae and the corresponding barrels a one-to-one relationship has been demonstrated electrophysiologically[23,29,43,44]; in lesion experiments (conventional histology[35], cytochrome oxidase[47]); and in deoxyglucose studies[21]. Given the localization of supernumerary whiskers (SWs) at sites on the whiskerpad that in the embryo correspond to the medial and the lateral lines of fusion between the medial and the lateral nasal fold, and between the latter and the maxillary arch (see end of Discussion), it has been proposed[37] that the pattern of the follicles is responsible for the establishment of the pattern of their central representations (see also Killackey[16] and Van der Loos and Dörfl[36]). Variations in number of mystacial vibrissae in mice were reported earlier[4,6,15,51]. In a recent paper[39] we described deviations from the standard pattern of mystacial vibrissae in a population of ICR mice and in several inbred strains and suggested that there is a genetic basis for the occurrence of SWs. The present paper is an attempt to prove this point.

Starting with an initial population, part of which figured among the ICR population described recently[39], we bred mice for various patterns of mystacial vibrissae and thus established 23 strains that are currently maintained. There are four classes: I, a strain bred for the standard pattern; II, eight strains bred for bilateral SWs at a given site or at given sites (the so-called fixed strains); III, four strains bred for a maximal number of SWs in circumscribed regions of the whiskerpad; and IV, 10 strains bred for an asymmetrical distribution of SWs.

Van der Loos et al.[38] and Van der Loos and Welker[40] report some results of breeding of strains of all classes. The present article is about the results for classes I, II, and III.

The strains of mice described here are important in the context of two general aspects of biology. The first is pattern formation. Inspired by our results, we propose two sets of genes that determine the pattern of mystacial vibrissae. The second aspect relates to developmental neurobiology; the question here is: how does the nervous system, peripheral and central, adapt itself to the presence of an increased number of peripheral somatosensory organs? We address this point in a paper in preparation; see also Van der Loos and Welker[40].

Unlike other neurological mutants (for a review see Hall et al.[13]) ours are normal mice except for the fact that they are "enriched" by SWs. In 1925, Danforth assigned to vibrissae the property of "phylogenetic individuality" based on their numerical constancy[4]. We have asked ourselves whether the variation in whisker patterns between our strains would not lead to a reassessment of Danforth's concept.

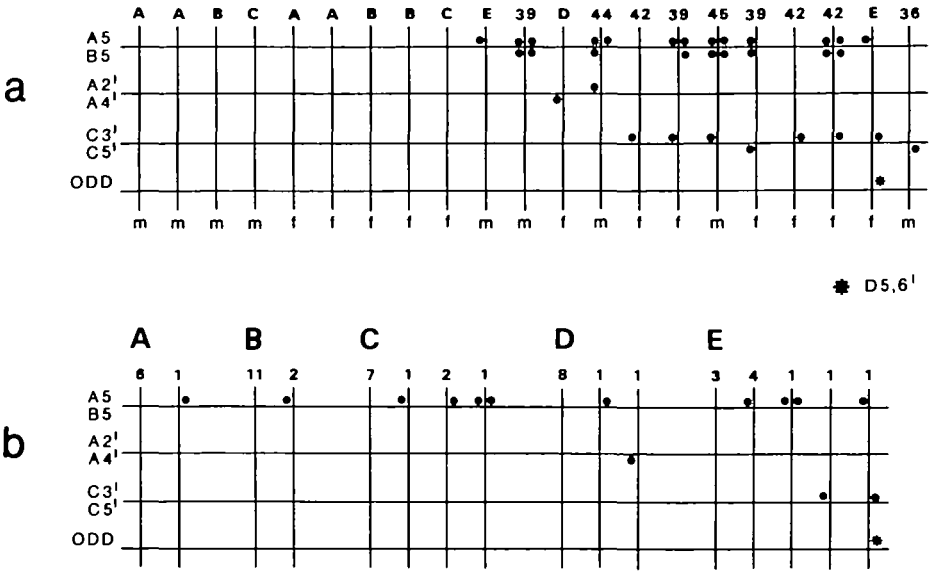


FIGURE 1 Graphic representations of (*a*) the population of animals that served as "Source" of the mouse strains whose development is described in this paper, and (*b*) the five litters of animals of the ICR stock from which mice were taken to establish the "Source" animals, and that have not been described in our previous report[39]. In *a* each individual mouse is symbolized by a vertical line, and each supernumerary whisker (SW) by a dot. A dot to the left of the vertical line signifies a SW on left whiskerpad; a dot to the right, on the right pad. Columns to the left hand side of the staffs give the sites at which SWs occur. Vertical bars without dots represent animals with the standard pattern. The number above a vertical line denotes the litter from which the animal was taken; it corresponds to the litter designation in Figure 3 of Van der Loos et al.[39]; a letter above a vertical line corresponds to a litter displayed in *b*. The letters *m* or *f* below the vertical bar denote sex. In *b*, the animals are grouped in litters (A-E) according to phenotype, i.e., the distribution of SWs over the two whiskerpads of the corresponding mice. Here, a vertical line symbolizes a phenotype. The number at the top of each line signifies the number of individuals possessing that particular phenotype. In *b*, no distinction was made regarding sex.

Materials and Methods

At the origin of the 13 strains of mice described were 21 animals, together referred to as the "Source." Of these, nine (four males and five females) were among the offspring of 45 ICR females obtained from the Institut für Zuchthygiene, University of Zürich. Characteristics of these offspring (597 animals) have been described by Van der Loos et al.[39]. In Figure 1*a*, these nine mice, which we call group 1, are identified by numbers corresponding to those given to the litters characterized in Figure 3 by Van der Loos et al.[39].

Twelve other mice (five males and seven females)—group 2—were among the offspring (51 mice) of four additional ICR mothers from the Zürich Institute, one of which was received pregnant, and three of which were fertilized in our laboratory without aiming for a particular whisker pattern. In Figure 1*a*, the 12 mice of group 2 are identified by letters corresponding to the code names (A-E) of the litters used in Figure 1*b*. The 51 mice in question are represented following the convention adopted for Figure 3 in Van der Loos et al.[39] and, together with the 597 mice described earlier, form a group of 648 animals—the "initial population."

In fact, except for one case in group 2, where the father of a litter (litter D in Figure 1*b*) had a standard pattern, no parental whisker configuration was known to us. Likewise, the relationships between parents of groups 1 and 2 were unknown except that, until the Source was generated, brother-sister mating was avoided.

Early in the breeding program, family lines were not kept separated. We then still did not

know for which whisker patterns it was possible to select. In this initial phase we analyzed, of the strains in class II, the combined offspring (F_1) of all couples of which both mates had the desired phenotype. These data are presented as the "A generation" (GEN A in Figure 6). This phase of the breeding period was terminated at arbitrary times that varied from strain to strain. Then the strains were named and the F_1 from the admitted parents formed the first generation (GEN 1). The strains were kept separated except for a few interstrain crosses.

The animals were kept in a room of 4 × 2 m and 3.4 m high, in 15 cm high plastic cages of 20 × 24 or 12 × 24 cm. Food (mouse-and-rat pellets "850," Nafag AG, 9202 Gossau, Switzerland) and water were given ad libitum. Typically, the cages housed one male and usually two females. Females à terme were placed alone, but kept with their mate when a second or third litter was desired. Temperature was adjusted to about 25°C. Humidity was maintained at about 50 percent. The room was artificially lit from hours 0600 to 1800.

Cages were inspected for newborns every day and screening for vibrissal pattern took place within 24 hours after birth. Litters were reduced to 10–12 animals (mean litter size was ca 10 pups). Two days after birth, animals with undesired whisker patterns as well as eventual runts were eliminated so that a mother stayed with 5–8 pups. A third screening for overall quality of the animals took place when the young were 21–28 days of age; they were weaned and new couples were formed. The total number of animals eliminated was 400/week; pups up to 2 days of age were anesthetized and killed by cooling; adults were killed by rapid cervical dislocation.

For the project at hand, about 15 new matings were made per week, (defined by the number of females) and an equal or smaller number of litters were born. Gestation was 19–20 days; typically, females had their first litter at 45–70 days of age. Brother-sister mating was practiced when possible. Sometimes one or both parents were mated with one or more pups, or lines within one strain were mixed.

Complete brother-sister mating was started at different times for different strains. Thus, one strain consisted of several lines; as we amplified those with the best record. Initially, the colony was plagued by frequent illnesses: hair loss, often accompanied by eczema; and dehydration through diarrhea followed by sudden death. The hair loss was caused by a ca 1 mm long white tick living preferentially in the perioral skin; it was treated by a dip-cure of veterinary quality bromociclen (Alugan, Hoechst). The diarrhea was caused by an intestinal parasite; it was treated by adding veterinary quality oxytetracycline chlorhydrate (Tetramycin, Pfizer) to the drinking water for one week. Although some strains showed minor differences in characters such as eye-, ear-, tail- and body-size and exploratory behavior, the animals were healthy and without obvious defects.

The total number of animals screened in the context of this study was 29 471. Screening of mice meant evaluating the whisker pattern in newborns, a procedure we reported[39], together with a description of our nomenclature for standard and supernumerary whiskers. For the analysis we chose a number of generations totalling 16 278 mice, the last generations being those completed in June 1984.

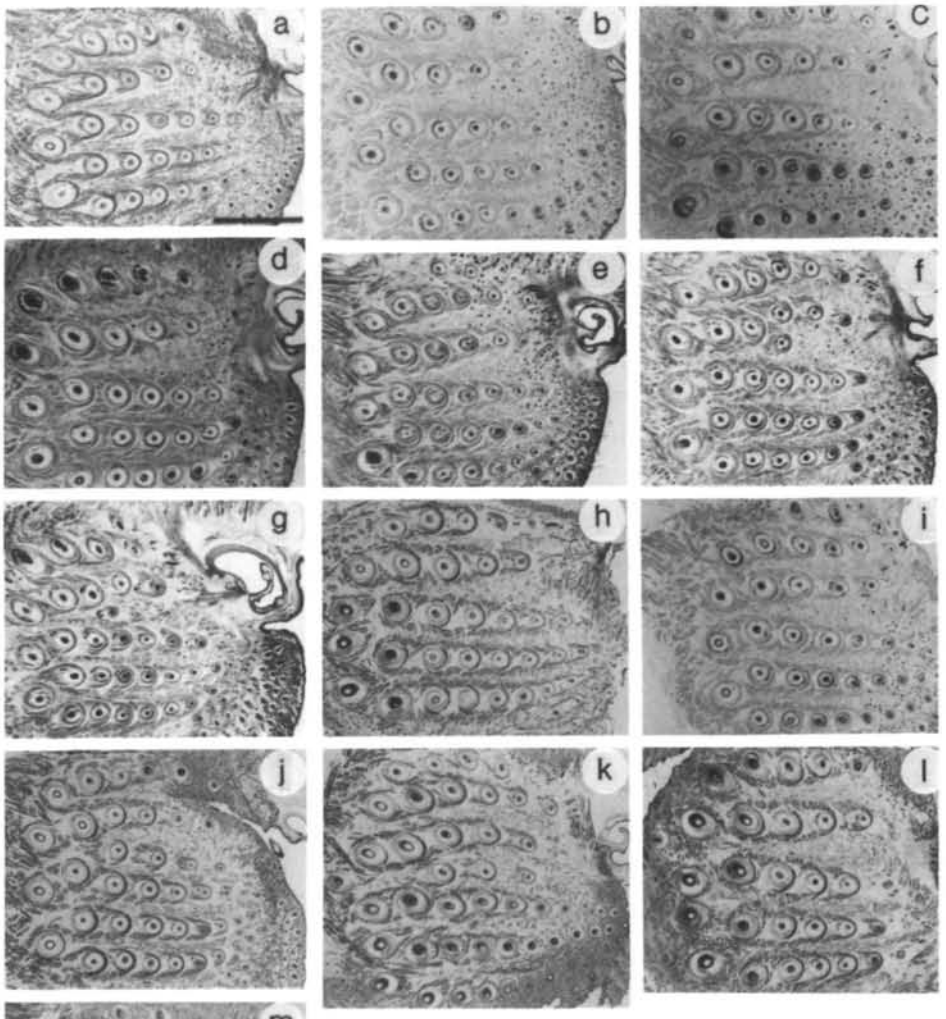


FIGURE 2 Photomicrographs of right whiskerpads of adult mice illustrating the pattern of mystacial vibrissae of the 13 strains described. Magnification is the same for all pictures (bar in *a*: 2 mm). Rostral is to the right; mediodorsal, to the top. For identification of both standard and supernumerary whiskers, see Figure 3. *a*—NOR, *b*—A/A, *c*—H/H, *d*—AP/AP, *e*—AAP/AAP, *f*—CP/CP, *g*—AC/AC, *h*—D/D, *i*—MAP, *j*—MCP, *k*—M/M, *l*—CoDo, *m*—MBP. Whiskerpads are cut tangential to the surface of the skin at 15 μm (*a, b, c, h, i, j, k, l, m*) or at 40 μm (*d, e, f, g*).

Some SWs among those observed in this study were not present in the initial population: B' medial to row B (i.e., between rows A and B); Co between "straddlers" β and γ; Do between γ and δ; and a double straddler, double β, with two vibrissae issuing from the same orifice.

We report on three of the four classes of strains mentioned above (see Figures 2–4).

Class I

NOR, bred for the normal whisker pattern as defined earlier[39].

Class II

A/A, bred for having on both sides an A5 only.

H/H, bred for A5 and B5 whiskers on both sides. Mice with other SWs were excluded.

AP/AP, bred for the bilateral presence of one SW in the part of the skin corresponding to the medial line of fusion. The SW, an "A'," had to be in a particular site: A3,4', i.e., medial to a position halfway between A3 and A4 (some animals were taken with an A4'). A5, often present, was allowed, other SWs were not.

AAP/AAP, bred for two A's at both sides. A5 and B5 were allowed; other SWs were not.

CP/CP, bred for the bilateral presence of one SW in the C3' position in the lateral line of fusion, i.e., dorsomedial to C3. A5 and B5 were allowed, other SWs were not.

AC/AC, started by breeding animals with

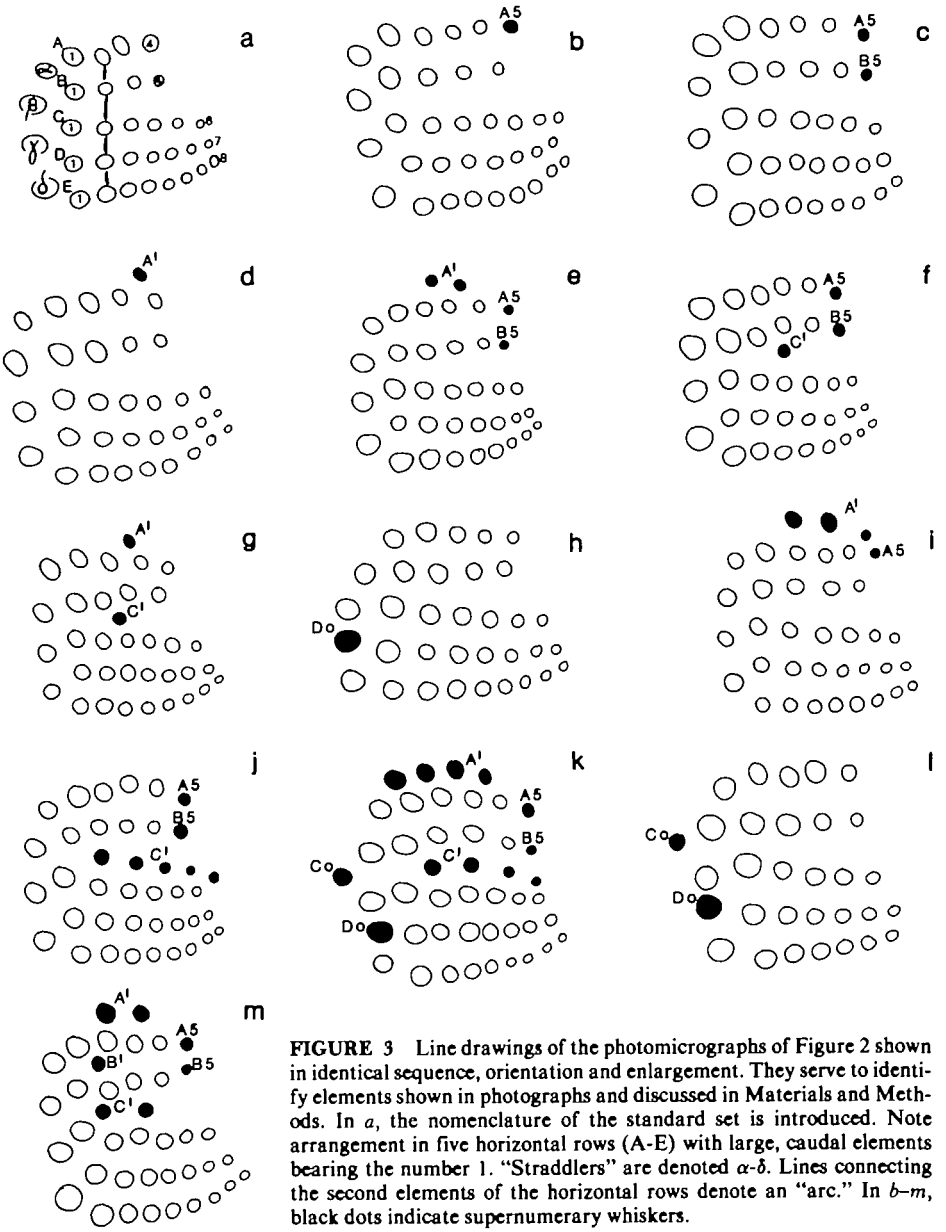


FIGURE 3 Line drawings of the photomicrographs of Figure 2 shown in identical sequence, orientation and enlargement. They serve to identify elements shown in photographs and discussed in Materials and Methods. In *a*, the nomenclature of the standard set is introduced. Note arrangement in five horizontal rows (A-E) with large, caudal elements bearing the number 1. "Straddlers" are denoted α-δ. Lines connecting the second elements of the horizontal rows denote an "arc." In *b–m*, black dots indicate supernumerary whiskers.

one A' plus *one* C' whisker to one side, and preferably accompanied on the other side by either an A', or C', or both. This phenotype was first observed in other strains, for example in M/M (see below); and occurred as if by accident. A5 and B5 were allowed. Throughout the generations there was the tendency for animals to have more A' and C' whiskers than desired. There was no GEN A phase.

D/D, bred for bilateral presence of two whiskers: "double γ" or "Do" (Figure 5). Double γ apparently arose from one follicle. Do, often in line with the D row, was a whisker at a discrete site somewhere between γ and δ. No distinction between Do and double γ was made but animals with a discrete Do were preferred. No other SWs were allowed. There was no GEN A.

For the display of the breeding results of the strains of groups I and II similar conventions were used (Figure 6). Per generation, or per set of subsequent generations when one had less than 100 individuals, the animals were grouped according to phenotype. For each strain, the frequencies of the phenotypes in the initial population were calculated and data were presented as for the generations.

Class III

MAP, bred for a maximum number of A's on both sides with disregard for location. A5 and B5 were allowed; other SWs were not allowed. The first generation is the offspring of mice from the AAP/AAP strain that had more than two A's on one or both sides.

MCP, bred for a maximum number of C's on both sides. We capitalized on the tendency of SWs in the lateral fusion line to increase. A5 and B5 were allowed; other SWs were not.

M/M, bred for a maximum number of SWs regardless of position. The strain was established early in the program from various lines by mating mice having many SWs. A litter scored high when many siblings followed that tendency; the best were used to start inbreeding. Early on, when a fair number of couples came from different lines, A's predominated in some litters while C's did in others.

The presentation (Figure 7) of the breeding results for class III is different from that for classes I and II. Per generation animals were classified according to the number of SWs found on both sides: for MAP the total number of A's; for MCP, the total number of C's; for M/M, all SWs taken together. Neither A generations nor frequency distributions of the initial population were created. For each generation, animals bearing many SWs were classified as to sex. There was no correlation between SW number and sex, and we ceased further analysis on this point.

In addition, we present two strains started more recently (1981).

CoDo. We took animals from lines bred for Co whiskers, found in strains with C's. These mice were crossbred with those having Do whiskers (mainly D/D). Promising offspring were taken for inbreeding. C's, commonly accompanying the Co and Do whiskers, tended to disappear subsequently.

MBP. Occasional B' whiskers were observed in animals from the M/M, H/H, and AC/AC strains, as well as in strains bred for an asymmetrical distribution of C' whiskers. Mice were mated for enhancing the number of B's within the population, regardless of occurrence of other SWs.

For the presentation of these two strains (Figure 8) we selected the best litter from the most recent completed generation and traced back its origins. The quality of a CoDo litter was expressed as the number of Co, Do combinations per side, compared with the total number of animals in the litter. For MBP, we used the number of B' whiskers compared with the number of animals per litter.

For histology, adult animals from the different strains were anesthetized with sodium pentobarbital (Nembutal, Abbott) i.p. and perfused with 10 percent neutralized formalin in 0.9 percent NaCl. Brain and whiskerpads were carefully disected out and postfixed in the same solution. The brain, after dehydration, was bisected sagittally and embedded in celloidin; the hemispheres were cut tangentially to the pial surface over the barrelfield[27]; whiskerpads were placed in 20 percent sucrose, then cut in a cryostat. The brains were Nissl-stained for cell bodies (methylene blue); whiskerpads were stained for axons[3], or for myelin according to a modified Lillie method (Cruz et al., ms. in prep.)

Results

Improvement

Improvement had two aspects: the relative increase in the number of animals with the desired character (enhancement) combined

with the relative decrease of the number of animals with undesired characters (deletion).

Class I consisted of one strain:

NOR (Figures 2, 3, and 6*a*) having the standard whisker configuration on both sides, was unique in that such animals predominated in the initial population (336 out of 648). Nine out of the 21 animals of the Source had this phenotype. At the 7th and 8th generation an enhancement to 95 percent was reached, even without a passage through a "GEN A" stage. A' and C' were rare; A5 was difficult to delete. Most elements marked "ODD" were SWs among the straddlers; some mice lacked an A4.

Barrelfields appeared to be identical to those from "standard" mice of the initial population (see Figure 1 in Van der Loos et al.[39]).

Class II consisted of eight strains (for CoDo see end of section "*Improvement*"):

A/A (Figures 2, 3, and 6*b*). A5 was the most frequent SW in the initial population. Although 61 out of its 648 animals had the A5/A5 phenotype, none of these figured among the Source. The phenotype reemerged early in the breeding program. Mice with the desired pattern were mated and the first results were grouped as GEN A; the A5/A5 phenotype reached 35 percent. After a steady improvement, 90 percent was reached at GEN 13+14, while very few animals had undesired characters. The most frequent contamination was an A'.

Barrel A5 (Figure 4*a*) fitted between A4 and the small barrels associated with the "rhinal vibrissae"[51].

H/H (Figures 2, 3, and 6*c*). B5 was the second most common SW in the initial population. Two B5s plus two A5s were seen in six of these animals, one of which became part of the Source. From GEN 6+7 onward, more than 95 percent of the animals had the desired character. The most common deviation was the lack of a B5, usually on the right. GEN A had more than three times as many C's as A's; they subsequently all but disappeared.

Barrel B5 was in the small field anterior to B4 that, in NOR, is devoid of barrels, and lies between the C row and the barrels of the rhinal vibrissae. The location of the A5 barrel was as in A/A.

AP/AP (Figures 2, 3, and 6*d*). This strain, present in the initial population but not in the Source, was one of the least successful. Although 80 percent of its animals had the desired character in GEN 6+7, a decline in quality then set in and the generations became smaller (through infertility and illness) forcing the use of animals from low-quality litters. The most frequent site was A3,4'. The tendency toward one or no A's was more difficult to eradicate than that to have too many. With the decline in enhancement after GEN 6+7 there was a less successful deletion of C' whiskers.

In GEN 1, 141 mice shared 229 A5 whiskers and 52 B5 whiskers; for GEN 12–15 (140 mice), these numbers were 267 and 0, respectively.

The A' barrels were lateral to the A row at places corresponding to those of the A' whiskers; they protrude beyond the limits of the standard barrelfield. The adjacent barrels of row A may be compressed.

AAP/AAP (Figures 2, 3, and 6*e*). This phenotype, not present in the initial population, rapidly became stabilized at about 75 percent. Most A' whiskers were A3,4' and A2,3'. The tendency towards adding A' whiskers remained; that for animals with too few A's decreased. C' vibrissae were deleted.

In GEN 1, 243 mice shared 421 A5 and 170 B5 whiskers; for GEN 16 (116 mice), these numbers were 176 and 45, respectively.

The barrelfield was comparable to that of AP/AP; here there were *two* A' barrels lateral to row A.

CP/CP (Figures 2, 3, and 6*f*). In the initial population there were two animals with this phenotype; they formed part of the Source. The strain appeared to be improving, although the last generation reported is not the best. The tendency to have one C' has been difficult to delete. A's and "oddities" (SWs among straddlers), frequent in GEN A and GEN 1, diminished. The most frequent C' was C3'. The few lines with C5,6' died out after two generations.

In GEN 1, 471 mice shared 913 A5s and 838 B5s; for GEN 18 (133 mice) these values were 225 and 236, respectively.

The C' barrels lay in their "proper" places, between rows B and C.

AC/AC (Figures 2, 3, and 6*g*). Animals of this phenotype did not occur in the initial

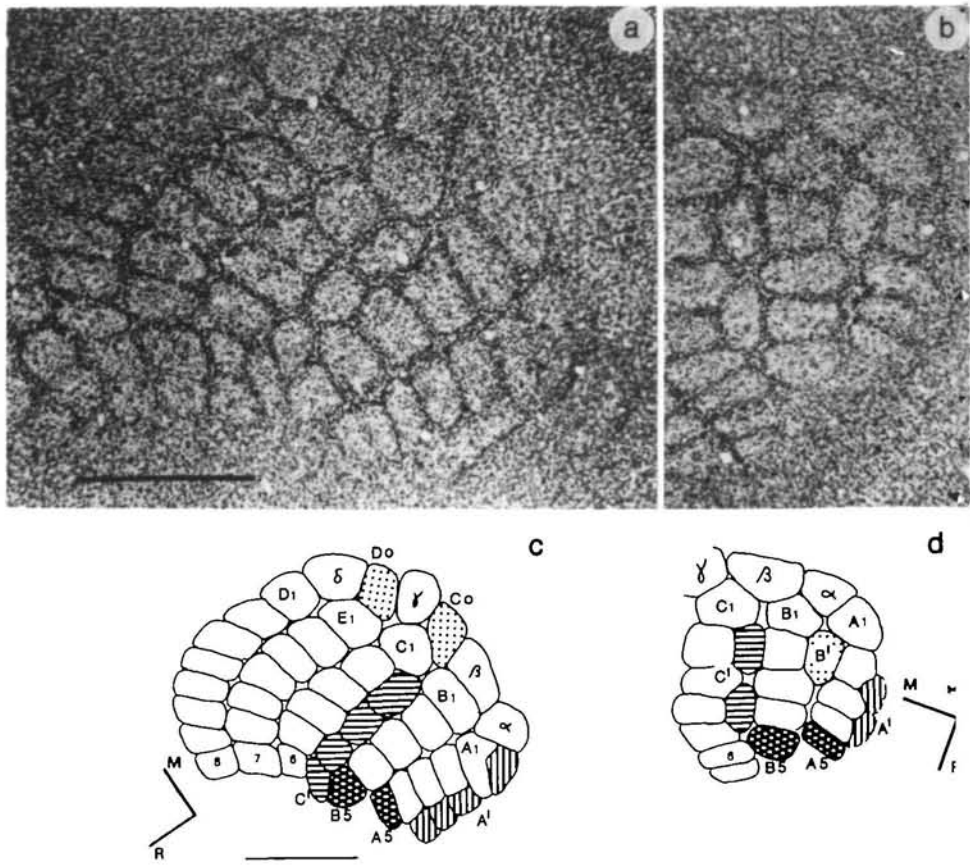


FIGURE 4 Two collages made from photomicrographs of serial sections through left barrelfields of a mouse from the M/M strain (*a* corresponds to whiskerpad shown in Figure 1*k*) and of an animal from the MBP strain (*b* corresponds to whiskerpad in Figure 1*m*). Barrelfields were cut at 40 μm tangential to the surface of the cortex, and stained with methylene blue. In *a* the whole barrelfield is shown, whereas in *b* only the part surrounding the B' barrel. Bar in *a*: 500 μm; same magnification for *a* and *b*. *c* and *d* are drawings of the barrelfields in *a* and *b*; the orientations correspond; R = rostral, M = mediodorsal. Bar in *c* pertains to *d* as well and represents 500 μm.

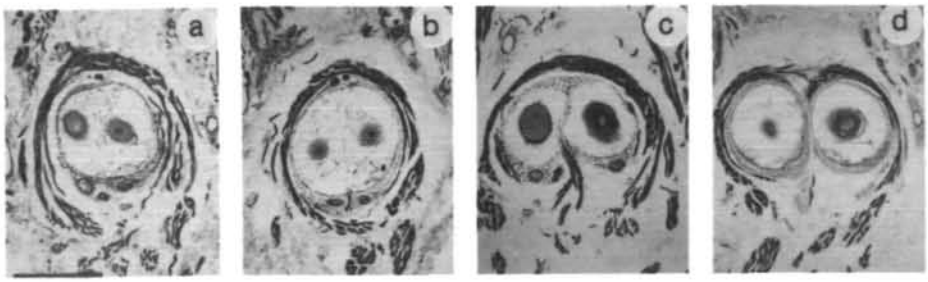


FIGURE 5 Four variations of whiskers at the Do site. Photomicrographs were from 15 μm sections taken parallel to, and at comparable distance from, the skin surface and stained by a reduced silver method. Bar in *a* pertains to all photographs and represents 50 μm; for all photos rostral is up. *a* shows a vibrissa of the double γ type; there are two whiskers placed in one undivided sinus. *b*—same as *a* but sinus shows signs of division into two compartments. *c*—two vibrissae, each in a separate compartment, both of which form part of a single follicle; at deeper levels, the two compartments merged while the vibrissae themselves remained separate. *d*—complete separation of follicles, one of which was identified as γ, the other as Do.

population. There was one with an A',C' combination unilaterally, which did not form part of the Source. This strain has been difficult to improve. In GEN 6+7 most animals lacked one or more of the desired SWs, while in GEN 17 most animals had an excess of A's or C's. Interestingly, the sites where A's and C's most occurred were the same as those observed in strains AP/AP and CP/CP. Although SWs among the straddlers did not form a selection criterion, their frequency increased: in GEN 1 (n = 235) there were 11 SWs and in GEN 17 (n = 165), 72. For the latter generation, Co was the most frequent SW among the straddlers (93 percent), the remaining ones being double β's. There were no Do whiskers. Two mice in GEN 1 had a B', a contamination not present in GEN 17.

In GEN 1, 235 mice shared 238 A5s and 256 B5s; for GEN 17 (165 animals) these numbers were 323 and 325, respectively.

In both whiskerpad and barrelfield this phenotype led to "arcs" of seven whiskers and of seven barrels where normally there are five (Figure 3*a*).

Do/Do (Figures 2, 3, and 6*h*). One animal with only one double γ was seen in the initial population; it did not form part of the Source. Although rare elsewhere, Do and double γ were extremely successful in this strain: animals with one or two Do's were numerous already in GEN 1. In GEN 10+11 75 percent of the animals had the desired phenotype. Contaminations, which were of all types, disappeared with the enhancement. Variations at the Do site showed a continuum between a distinct Do whisker and a configuration called double γ: two whiskers from one follicle (Figure 5). These configurations led to local crowding of vibrissae.

For a Do whisker, the barrelfield showed a barrel between γ and δ, whereas a double γ appeared to be represented by an oversized γ barrel.

Class III consisted of four strains (for MBP see end of section "*Improvement*"):

MAP (Figures 2 and 3). In the initial population the maximum number of A's in one animal was two—one on each side (not included in the Source). Figure 7*a* shows the enhancement: the mode of the frequency distribution of animals classified according to number of A' whiskers shifted from 4 to 5; the range, from 2-6 to 4-8.

With the increase in number of A's over the generations, their distribution changed over the territory of the medial fusion line. The left top pair of histograms of Figure 9 (A'-MAP) allows one to compare the occupation of this territory by SWs in GEN 1 and in GEN 12+13, and for left and right whiskerpads. In all cases, A3,4' was most frequent, followed by A2,3'. Only later did more rostral and caudal locations become more occupied. The percentage of animals with one or more C's (considered contaminations) increased from 1.2 percent for GEN 1 to 7.2 percent for GEN 14. But for A5, B5 or C' whiskers, no SWs were observed in GEN 1 and 14.

In GEN 1, 180 mice shared 240 A5s and 89 B5s; in GEN 14 (167 mice), these numbers were 320 and 228, respectively.

The A' barrels occupied predictable places as described for AP/AP.

MCP (Figures 2 and 3). In the initial population the maximum number of C's in one animal (not part of the Source) was four—two on each side. Between GEN 1 and 16 the mode of the distribution of animals classified according to the number of C's, shifted from 4-5 to 10; the range, from 0-9 to 6-12 (Figure 7*b*).

The right top pair of histograms of Figure 9 (C'-MCP) permits comparison, for GENs 1 and 12, of the frequency of vibrissae at sites along the territory of the lateral fusion line. While in GEN 1 C3' was preferred, C2' was equally present in GEN 12 when, also, all sites of GEN 1 were more frequently occupied, but no new ones added.

The percentage of animals with A' whiskers increased from 5.7 percent for GEN 1 to 18.8 percent for GEN 16. The percentage of mice possessing SWs among the straddlers demonstrates a greater increase: from 6.0 percent for GEN 1 to 62.4 percent for GEN 16. Among the latter contaminations, Co was most frequent. Its position is probably associated with the most caudal end of the lateral fusion line and one might argue that it should in fact be considered as a C' and not as a contamination.

In GEN 1, 384 mice shared 753 A5s and 711 B5s, while for GEN 16 (101 animals) these numbers were 200 and 200, respectively.

In the barrelfield the C's were found at places that corresponded to those of the C' whiskers, as described for CP/CP.

M/M (Figures 2 and 3). In the initial population the largest number of SWs per animal was six. There were two such animals, neither of which were part of the Source (see Figure 4 of Van der Loos et al.[39]). Figure 7*c* shows the considerable enhancement through the generations: the mode of the frequency distribution of mice classified according to the total number of SWs increased from 11 to 19; the range, from 1-15 to 13-24.

The bottom two pairs of histograms in Figure 9 permit the comparison, for GEN 1 and GEN 12+13, of the occupation of the territories of both fusion lines by vibrissae classified according to sites. Comparison between A'-MAP and A'-M/M and between C'-MCP and C'-M/M shows the similarity of the distributions of SWs in the two generations. However, there are differences for C2' and A2,3'.

Between GEN 1 and GEN 12+13 the number of SWs among the straddlers increased from 4 to 273. In GEN 1 (186 mice) they were at the Co or at the double β position; for GEN 12+13 (136 animals), the Co whisker was most frequent, followed by Do and some rare double γ's. Three animals of the first generation had one B'-whisker; in GEN 12+13, 24 mice shared 28 B's.

The 186 mice of GEN 1 shared 358 A5s and 317 B5s; for GEN 12+13 (136 mice), the numbers were 272 and 271, respectively.

Supernumerary barrels were found in places corresponding with those of the SWs, except for some rare cases in which no barrel could be found for a SW.

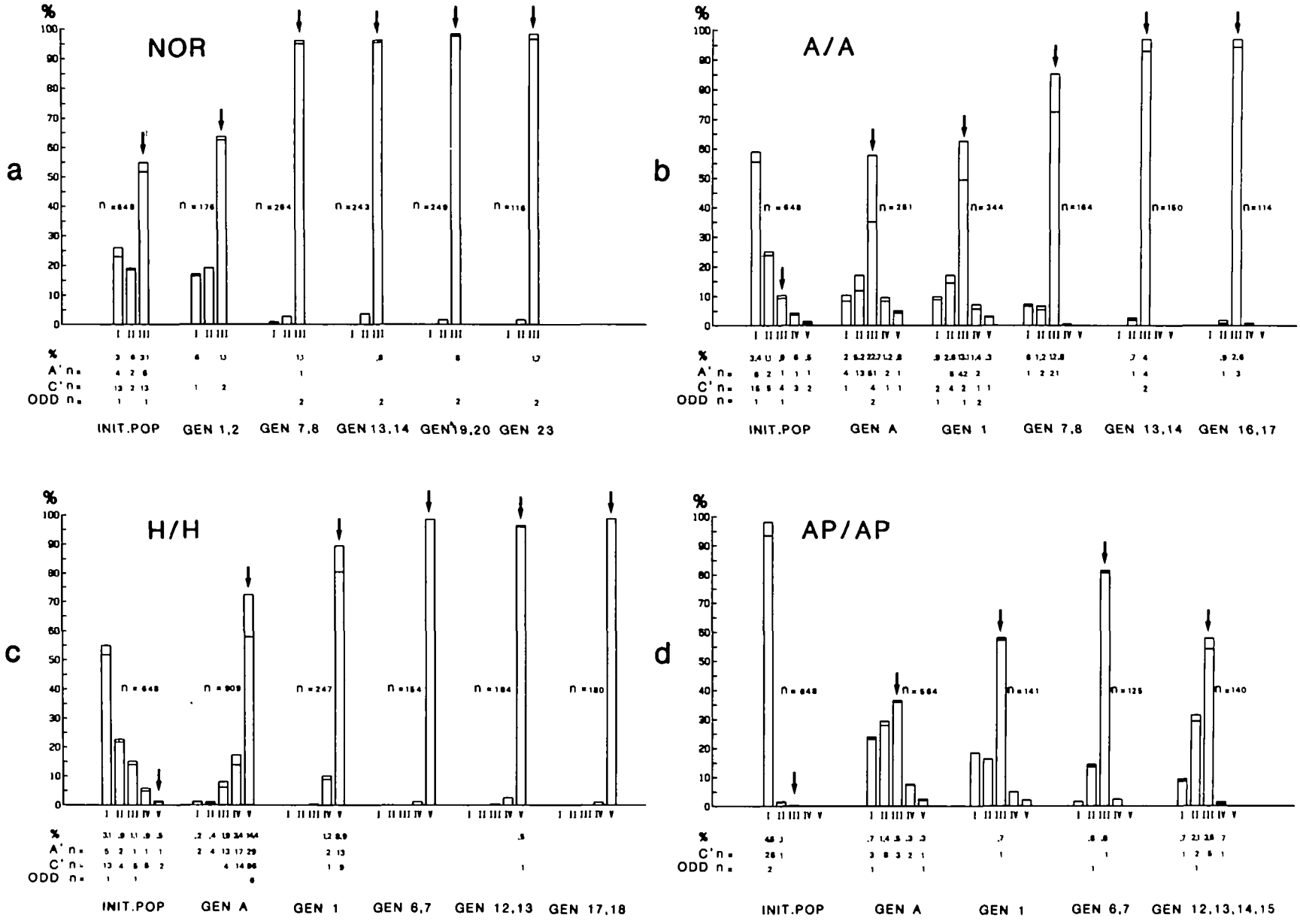
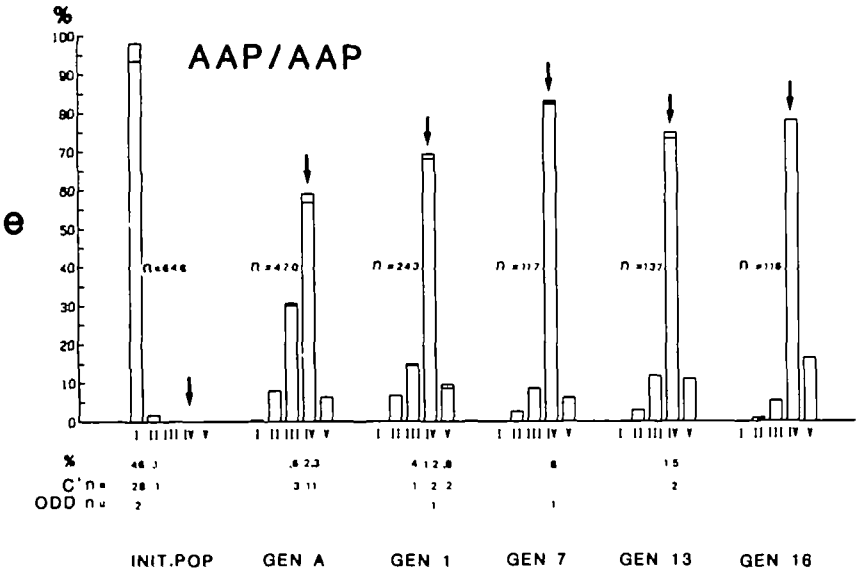
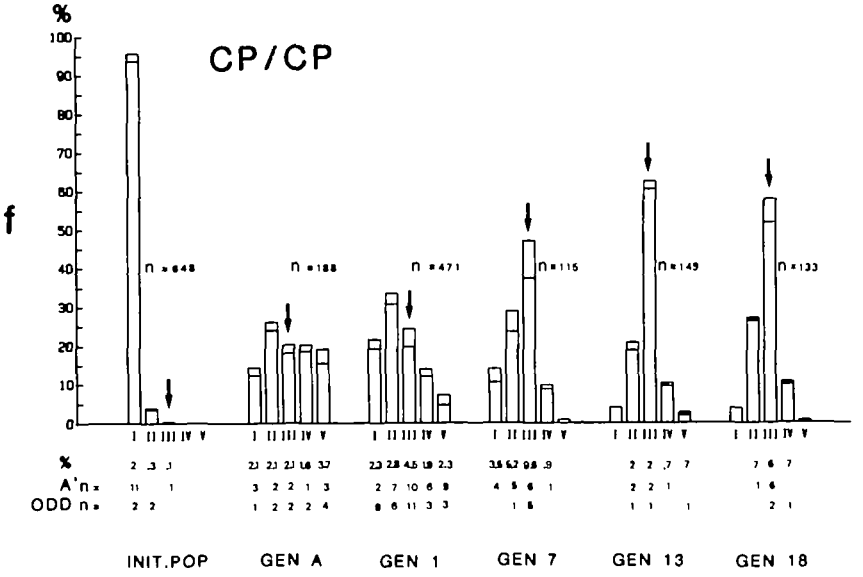
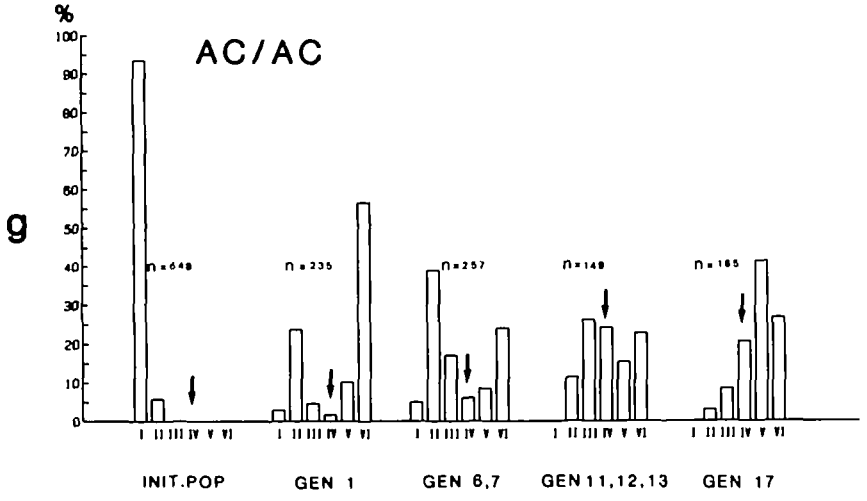


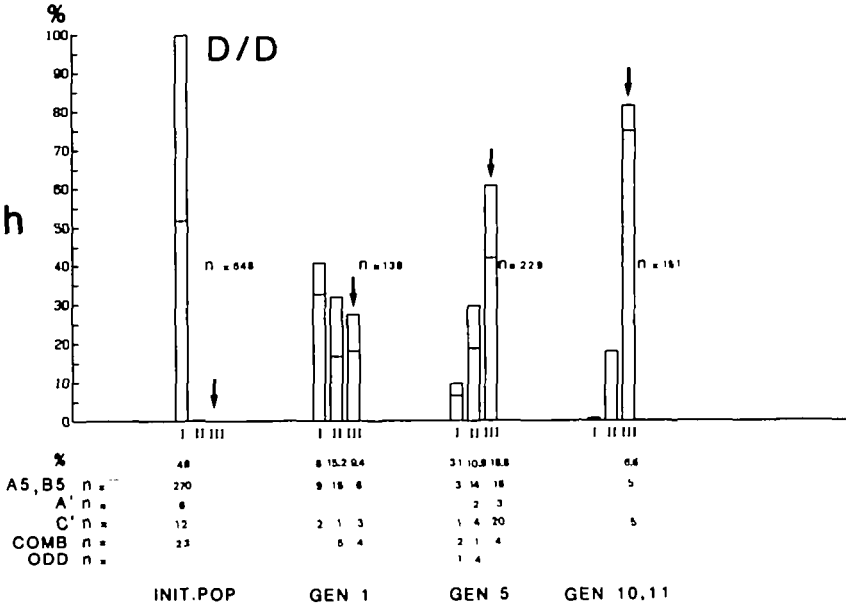
FIGURE 6 Improvement of eight mouse strains over several generations. One strain, NOR, was bred for the standard pattern (*a*); the others, for the bilateral presence of SWs at a given site or sites (*b–h*). See text for description of breeding criteria. For the initial population (INIT. POP), the A-generation (GEN A) and the subsequent generations, the animals are grouped according to phenotype marked by a roman numeral. Arrows point to the desired phenotypes. The percentage of each phenotype is represented by a bar. The "stacks" (upper parts of the vertical columns) represent two A5s (A/A); IV: two A5s and 1 B5; V: two A5s and 2 B5s. *c*—I: no A5s and B5s; II: 1 A5 or 1 B5; III: 2 SWs in sites A5 and B5; IV: 3 SWs in sites A5 and B5; V: A5,B5 on both sides (H/H). *d*—I: no A's; II: ≥1 A' on one side; III: 1 A' on either side (AP/AP); IV: 3 A's in total, distributed over both sides; V: ≥4 A's in total, distributed over both sides. *e*—I: no A's; II: 1 or 2 A's; III: 3 A's in total, distributed over both sides; IV: 2 A's on either side (AAP/AAP); V: ≥4 A's, of which 3 on one side. *f*—I: no C's; II: ≥1 C' on one side; III: 1 C' on either side (CP/CP); IV: 3 C' in total, distributed over both

animals with the phenotype in question but are contaminated by other SWs; their percentages are given as numbers immediately below the roman numerals. For the initial population as well as for each generation *n*'s are given and the total percentage represented by bars and stacks combined is 100% (ordinate). For each stack the contaminations listed to the left are expressed as absolute numbers, *ODD* meaning SWs among the straddlers, B's, and other oddities. For each strain phenotypes marked by the roman numerals are explained separately: *a*—I: one B5; ≥2 A5 and/or B5; II: one A5; III: the standard pattern (NOR). *b*—I: no A5s and ≤2 B5s; II: one A5 and ≤2 B5s; III: sides; V: ≥4 C's in total, distributed over both sides. *g*—I: no A's, no C's; II: ≥1 A' or ≥1 C'; or 1 A', plus 1 C' distributed over both sides; III: 1 A' plus 1 C' on one side, and ≤1 A' or ≤1 C' on the other side; IV: 1 A' plus 1 C' on either side (AC/AC); V: 1 A' plus 1 C' on one side, and on the other ≥2 A's or ≥2 C's, or ≥3 sites occupied by A's and/or C's; VI: on either side ≥2 A's; or ≥2 C's, or ≥3 sites occupied by A's and/or C's. *h*—I: no Do whiskers; II: 1 Do; III: 1 Do on either side (*D/D*). Double γ was counted as Do. "COMB" refers to animals having combinations of at least two of the three following types of SWs: A5 and/or B5, A' and C'.









CoDo (Figures 2, 3, and 8*a*). This combination of SWs among the straddlers did not occur within the initial population. The history of the litter placed on the right in Figure 8*a* started with a male of the first generation of the D/D strain and a female from a strain bred for an asymmetric distribution of C's and having two Co whiskers. During 13 generations a steady increase in the occurrence of the desired combination of SWs was observed.

MBP (Figures 2, 3, and 8*b*). The litter placed on the right in Figure 8*b* originates from animals, all with a B' whisker, from strains M/M and H/H, and a strain bred for asymmetric distribution of C' whiskers. The first crossings did not yield animals with B's, but subsequently these SWs appeared in increasing frequency.

Lateral asymmetries

To study the side preferences of SWs, we included data from all strains but CoDo and MBP. The analysis was done for the first and the last generation(s) of each strain, with the exception of MAP, MCP, and M/M, for which we presented the data of the generations that also were analyzed for the occurrence of SWs at one, or both, line(s) of fusion. The results are summarized in Table I and, for AC/AC and M/M, in Tables II and III.

For NOR, A/A and H/H, we determined the frequency of animals possessing an element, or elements, of the phenotype for which we selected: only on the left side, only on the right side, on both sides, or not at all. We found that side preferences expressed in the first generations are no longer present in the last. The higher frequency of animals having no SWs on the right in GEN 1 of NOR has its analog in a higher frequency of animals with A5 only on the left in GEN 1 of A/A, and of that of animals with A5+B5 only on the left in GEN 1 of H/H.

For the strains bred for A's (AP/AP, AAP/AAP, MAP), or for C's (CP/CP, MCP), animals were classified as having: 1) A's or C's on neither side; 2) more A's or C's on the left than on the right; 3) more A's or C's on the right than on the left; and 4) the same number of A's or C's on both sides.

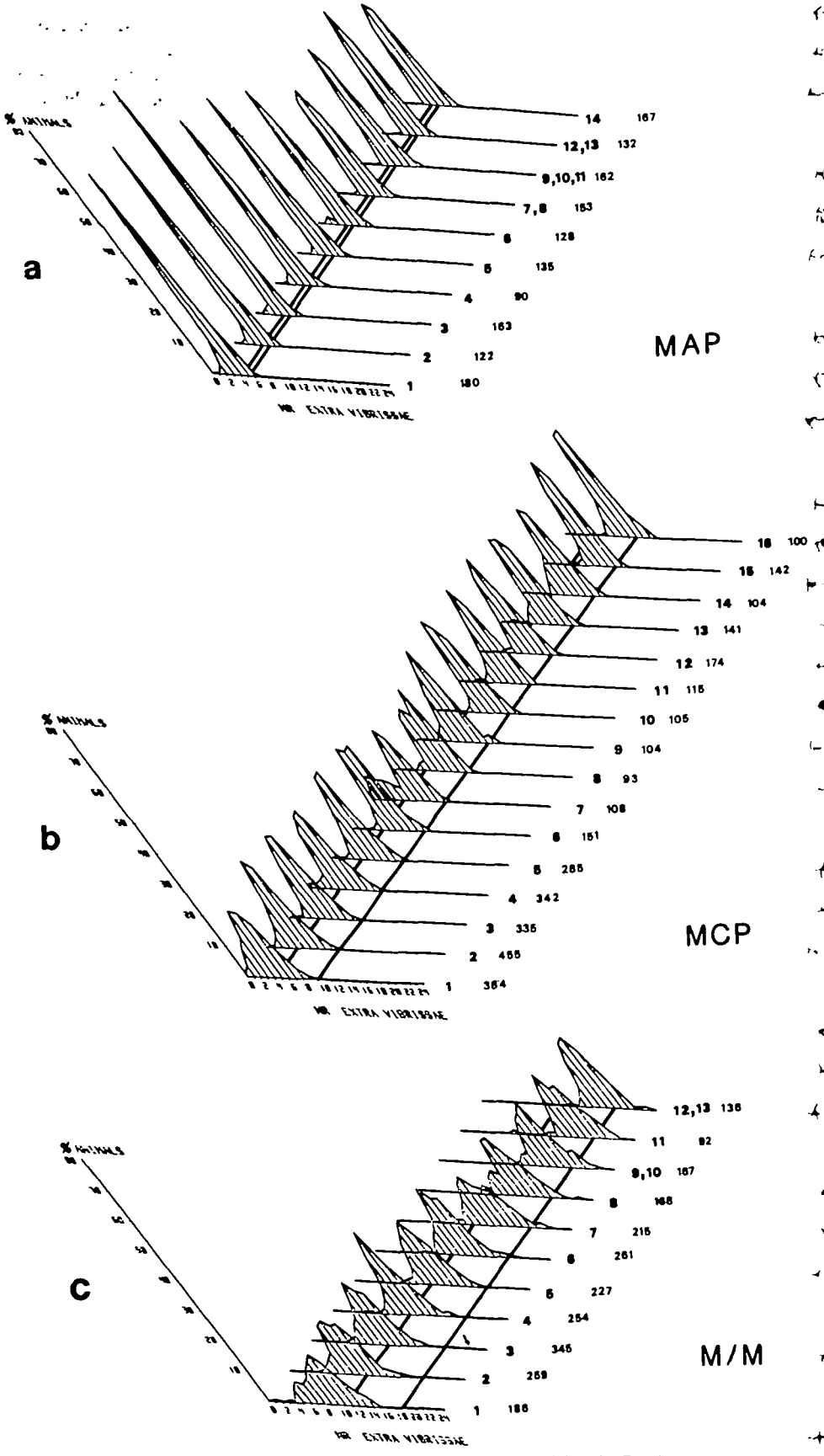


FIGURE 7 Demonstration of the success in breeding for (*a*) a maximum number of A' whiskers (MAP); (*b*) for a maximum number of C's (MCP); and (*c*) for a maximum number of supernumerary whiskers (SWs) irrespective of position (M/M). For each strain results are displayed as frequency distributions of animals per generation grouped according to number of SWs. Bold numbers to the right indicate generation or groups of generations; small numbers, numbers of mice per generation. Lines parallel to the Z-axis represent, from left to right, the mode of the frequency distribution of the first generation and that of the last generation analyzed. In *a*, animals are grouped according to the number of A's on both sides; in *b*, according to the number of C's; in *c*, according to the total number of SWs on both sides.

Side preferences for A's of AP/AP and AAP/AAP remained, while the percentage of symmetric animals stayed almost the same. As to MAP, the percentage of symmetric animals decreased in favor of animals with an asymmetric distribution. The preponderance of A's on the left is lower in MAP than in AP/AP and AAP/AAP.

C's have a right preference that decreased in CP/CP, but remained in MCP. The decrease of asymmetric animals together with the decrease in the percentage of animals without C' whiskers has its counterpart in an increase of the frequency of symmetric animals with C's. These changes are less prominent for MCP than for CP/CP.

In GEN 1 of D/D there was no preference for Do to occur on a particular side, but then a right preference developed accompanied by an increase of Do whiskers on both sides.

For AC/AC and M/M we had to group animals in 16 and 64 categories, respectively, in order to investigate whether some combinations of SWs showed a side preference. For AC/AC the classes were formed by the combinations of the four possibilities for the A'-whisker (analogous to those of AP/AP, AAP/AAP and MAP), and with the four possibilities for the C'-whisker (as for those of CP/CP and MCP). In M/M each of the 16 classes were subdivided into four, by taking into consideration the possible sites occupied by the A5s and B5s (as was done for H/H). For both strains, AC/AC and M/M, the expected values were calculated with the aid of contingency tables and compared with the observed frequencies. "Recent" SWs (B's and those situated among the straddlers) were not taken into account.

In GEN 1 of AC/AC, A' whiskers were mostly symmetrical, especially when there were no C's. Of the animals with asymmetric

Table I. Results of the analysis for laterality performed on two generations of the strains listed in the left hand column. Generations are indicated by number(s) in the second column. The numbers of mice per generation are in the right hand column. For each strain four categories of phenotypes were characterized in columns 1–4, which numbers indicate, per generation of each strain, the percentage of animals belonging to each category. Symbols between columns 2 and 3 reflect direction of the differences between the numbers placed in these columns. Left-right ratios (presented in column LRr) are defined as the numbers of animals with a preponderance of supernumerary whiskers (SWs) to the left, divided by those with a preponderance to the right. The symbols "bisecting" the indication of the phenotypes separate the phenotype on the left whiskerpad (left of the symbol) from that on the right (right of the symbol). For NOR, SW symbolizes all possible extra whiskers, hyphen meaning none; for A/A, A5 refers to A5 whisker, hyphen means no A5; in H/H, A5, B5 means the total number of SWs at the A5 and B5 sites on one side, hyphen meaning no SW at these sites; in AP/AP, AAP/AAP and MAP, A'L means the number of A' follicles on the left side, and A'R their number on the right, hyphen meaning no A' present; in CP/CP and MCP, C'L means the number of C' whiskers on the left side, C'R their number on the right, hyphen meaning no C's; in D/D, Do means the Do whisker, and a hyphen the absence of a Do

Strain	Generation(s)	Phenotypes 1	2		3	4	LRr	*n*
		SW/SW	-/SW		SW/-	-/-		
NOR	1	3.4	12.5	<	17.6	66.5	0.71	176
	23	0	1.7	=	1.7	96.6	1.00	116
		-/-	A5/-		-/A5	A5/A5		
A/A	1	9.8	12.2	≫	4.9	73.0	2.47	344
	16 + 17	0	0.9	=	0.9	98.2	1.00	114
		-/-	A5,B5 > A5,B5		A5,B5 < A5,B5	A5,B5 = A5,B5		
H/H	1	0	7.3	≫	2.8	89.9	2.57	247
	17 + 18	0	0.6	=	0.6	98.8	1.00	180
		-/-	A'L > A'R		A'L < A'R	A'L = A'R		
AP/AP	1	18.4	14.2	≫	7.1	60.3	2.00	141
	12 – 15	9.3	20.7	>	12.1	57.9	1.71	140
		-/-	A'L > A'R		A'L < A'R	A'L = A'R		
AAP/AAP	1	0	16.0	>	10.7	73.3	1.50	243
	16	0	12.1	>	8.6	79.3	1.40	116
		-/-	A'L > A'R		A'L < A'R	A'L = A'R		
MAP	1	0	15.6	>	14.4	70.0	1.08	180
	12 + 13	0	25.8	>	21.2	53.0	1.21	132
		-/-	C'L > C'R		C'L < C'R	C'L = C'R		
CP/CP	1	21.7	16.3	≪	34.6	27.4	0.47	471
	18	3.8	15.0	<	23.3	57.9	0.65	133
		-/-	C'L > C'R		C'L < C'R	C'L = C'R		
MCP	1	1.1	20.8	≪	42.7	35.4	0.49	384
	12	0	20.1	<	39.1	40.8	0.51	174
		-/-	Do/-		-/Do	Do/Do		
D/D	1	40.6	15.9	=	15.9	27.5	1.00	138
	10 + 11	0.7	6.6	<	11.3	81.4	0.59	151

A's, most had a left preference. To the contrary, C's had a higher incidence on the right. Symmetrical distribution of C's did occur, but mostly in the absence of A's. In the last generation we observed many animals symmetric for A' and C': 76 percent of the animals were symmetrical for A'; the remaining 24 percent had a strong tendency to the left, particularly in combination with a right preference for C'. Less than 50 percent of the animals were symmetrical for C' which, as in GEN 1, occurred mostly on the right.

For the generations of M/M analyzed we found A's to have a stronger tendency toward symmetry than C's. A's have a left, C's a right preference. For GEN 1, the side preference of A' was more frequent than expected among animals without C's or with C's symmetrically distributed. For side preference of C' a similar phenomenon was observed with respect to the absence of symmetrical presence of A's.

A5s and B5s were more valuable in GEN 1 than in the last generations in which only one animal out of 136 did not have A5+B5 on both sides. The variation in GEN 1 was similar to that in GEN 1 of H/H; for both there was a left preference for A5+B5 or B5. Asymmetry at these sites occurred far more often in animals without than in those with (asymmetric and symmetric) C's, while in animals with symmetric C's, A5s and B5s also were symmetric. For the recent generations, all animals were symmetric as to A5+B5 and all had C's. In contrast, a symmetric distribution of A's is not accompanied by a symmetric distribution of A5 and B5 whiskers.

Discussion

We presented data derived from a program of selective breeding for particular patterns of mystacial vibrissae in mice, carried out since 1977. The 21 mice that served as Source of the strains that we described came from an outbred ICR population.

What we call a whisker or a vibrissa is, in fact, a highly complex and mobile sensory organ, the whisker follicle, connected to the brain by a powerful nerve[17,42,45]. Such an organ, when supernumerary, was accompanied by an extra barrel at a topologically equivalent site in the barrelfield. But there were exceptions and they were found particularly in cases where "new" elements were involved, e.g., B' whiskers. With respect to the relationship between peripheral innervation and central representation, it appears that a threshold number of axons in a follicular nerve is needed for a barrel to come about (Welker and Van der Loos, in preparation; see also Welker and Van der Loos[45]).

Table II. Results of the analysis for laterality in strain AC/AC. The two numbers at the intersection of a column and a row are the percentages of animals of the first generation (left number, $n = 235$) and the 17th generation (right number, $n = 165$). Thus, a given phenotype is defined by combining the characters in a particular row and a particular column. A'L means number of A' whiskers on the left side; A'R, number on the right side. C'L means number of C' whiskers on the left side; C'R, number on the right side. Animals without A's on either side are indicated by "no A'L, no A'R," whereas animals without C' whiskers are placed in the column of "no C'L, no C'R"

	no C'L, no C'R		C'L > C'R		C'L < C'R		C'L = C'R	
no A'L, no A'R	3.0	—	5.1	0.6	9.8	—	9.8	1.8
A'L > A'R	5.5	—	2.5	1.2	6.8	9.7	4.7	7.3
A'L < A'R	8.5	—	3.0	1.2	1.7	0.6	3.8	1.2
A'L = A'R	17.0	—	2.5	13.9	11.1	30.3	5.1	32.1

Table III. Results of analysis for laterality in strain M/M. As in Table II, at the intersection between a column and a row, a phenotype is defined by the designations at the top of a column and at the left end of a row. In addition, the phenotype of each intersection is divided into four groups with respect to the occupation of the A5 and B5 sites: I, animals without A5 and B5; II, animals with more of these SWs on the left than on the right side; III, animals with less of these SWs on the left than on the right side; and IV, animals with an equal number of these SWs on each side. Thus per intersection the percentages of animals are given for four phenotypes, for the first generation (numbers to the left) and for the combination of the 12th and 13th generation (numbers to the right). Total number of animals in the first generation is 186; in the 12th+13th generation, 136. See Table II for further details of headings of rows and columns

		no C'L, no C'R		C'L > C'R		C'L < C'R		C'L = C'R	
no A'L, no A'R	I	—	—	—	—	—	—	—	—
	II	—	—	—	—	—	—	—	—
	III	—	—	—	—	—	—	—	—
	IV	—	—	—	—	0.5	—	1.1	—
A'L > A'R	I	1.1	—	—	—	—	—	—	—
	II	2.2	—	—	—	—	—	—	—
	III	—	—	—	—	—	—	—	—
	IV	2.8	—	5.0	3.7	6.1	12.5	6.1	16.2
A'L < A'R	I	—	—	—	—	—	—	—	—
	II	1.7	—	0.5	—	0.5	—	—	—
	III	1.1	—	—	—	—	—	—	—
	IV	0.5	—	5.0	4.4	3.9	3.7	3.3	2.9
A'L = A'R	I	—	—	—	—	—	—	—	—
	II	1.1	—	1.7	—	—	—	—	—
	III	2.8	—	1.1	—	1.7	—	—	—
	IV	6.6	—	7.7	16.9	22.6	17.6	16.0	21.3

The improvement for the strain of class I (NOR) indicates that the standard pattern is genetically determined. Improvement of the strains of class II and the increase in the number of SWs for the strains of class III demonstrate that selection for SWs is possible: the occurrence of SWs, too, has a genetic basis. Evidently, the genome encodes for the standard pattern of vibrissae and for various other patterns characterized by a certain number of SWs at defined sites.

Inequality in both degree and rate of improvement between the strains of classes I and II may be caused by differences in number and linkage of the genes involved. A rapid improvement, as attained in A/A, H/H, or NOR, suggests that relatively few genes are involved, permitting the early establishment of a high degree of homozygosity within the populations in question. Another possible explanation—not mutually exclusive—is that environmental factors play a less important role in the establishment of A/A, H/H, and NOR patterns than they do in that of the less successful patterns (CP/CP, AP/AP). However, rearing conditions for all strains were kept as similar as possible.

In addition to the so-called "fixed" pat-

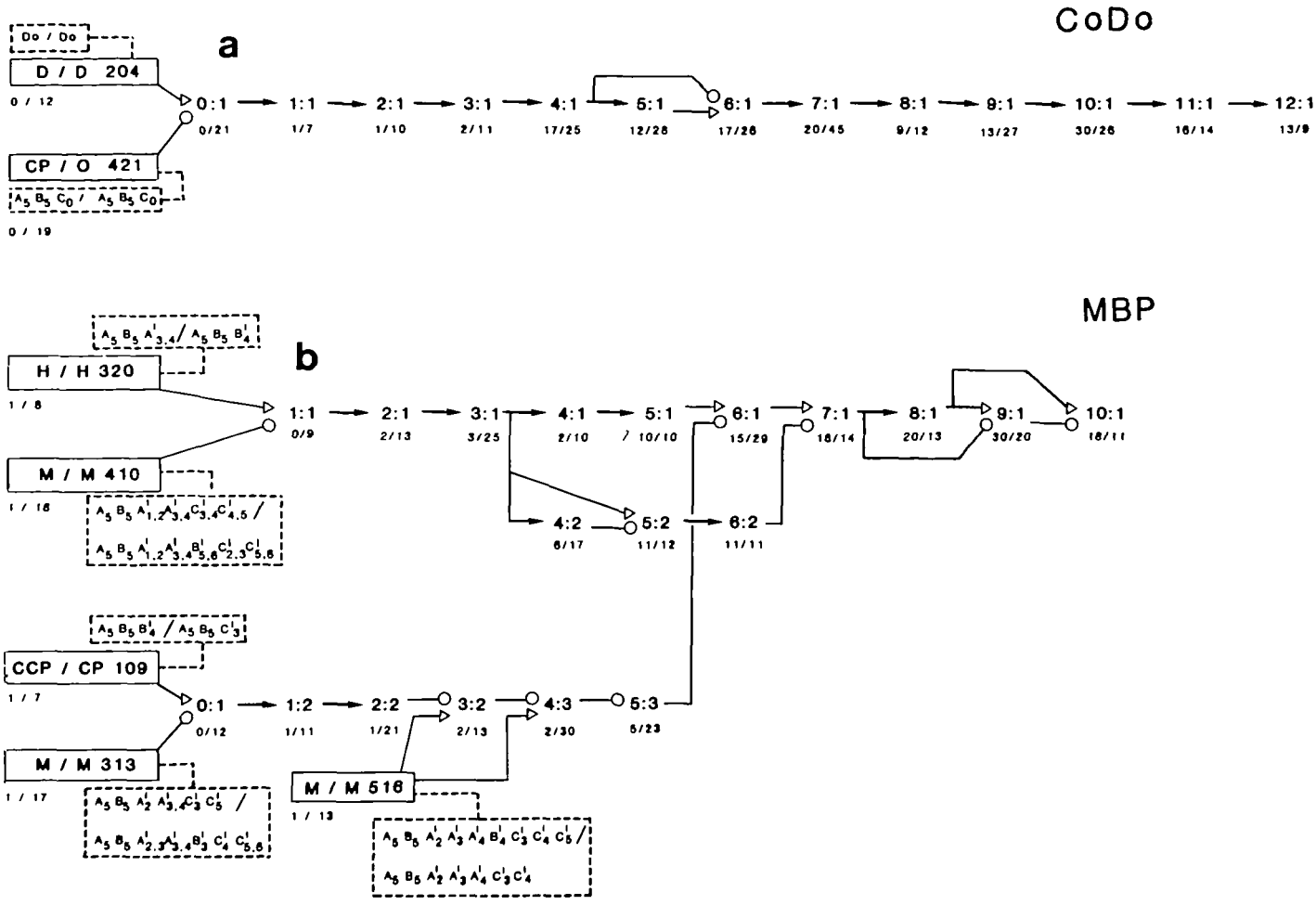


FIGURE 8 The graphical display of the family history of two recent litters of two different strains: in *a*, for CoDo; in *b* for MBP. These litters are placed at the right end of the "family trees." Each litter (i.e., the total offspring of one couple) is identified by heavy numerals: "generation number:litter number." Small numbers represent evaluations per litter: in *a* it is the number of Co,Do combinations per side over the total number of animals; in *b* it is the number of B' whiskers in the litter over the total number of animals. The animals at the origin of the two families are placed to the left; solid boxes contain the names of the strains in which the animals were born (for H/H, D/D and M/M see text; CCP/CP and CP/O are strains of mice bred for asymmetrical distribution of C' whiskers), as well as the generation in question (first digit of the 3-digit number) and the litter number (last two digits). The phenotypes with respect to supernumerary whiskers (SWs) of these "original" animals are identified in the boxes drawn with interrupted lines. The symbols to the left of the stroke correspond to SWs on the left whiskerpad; to the right, to SWs on the right whiskerpad. The way one generation gives rise to the next is symbolized by lines provided with one of three symbols: a solid arrow signifies that both parents came from same litter; when parents came from different litters the female is represented by a circle, the male by an open arrow. This complex background also illustrates the period of the breeding program from which we derived the A generations for strains of class II.

terns, we were able to select for a maximum number of SWs in one of the two lines of fusion, or in both; in fact, in MAP, MCP, and M/M the modes of the frequency distribution of SWs per generation are still increasing. For MAP this meant an increase in the number of A's, SWs related to the medial line of fusion; for MCP it was the number of C's related to the lateral line of fusion that increased; for M/M, A's and C's increased in number and so did "new" SWs, i.e., whiskers that did not, or hardly, exist when the breeding program started: B's and SWs among the straddlers. The increase in the frequencies of A' whiskers in MAP and of C' whiskers in MCP followed a similar sequence as in M/M. Thus, in M/M animals the sequence of the "filling in" of the lateral line of fusion did not seem to influence that of the medial line of fusion and vice versa.

For MAP and MCP a deletion of unwanted characters did not occur: both show a tendency to become M/M despite minimal use of "contaminated" animals for breeding.

From the more recent emergence of SWs among the straddlers (Co, Do, etc.) and of those medial to the B-row (B' whiskers) it became clear that we had not yet reached the limit of possible variations.

Danforth[4] argues that certain "organs of higher [species can be] thought of as in a certain sense descendants of corresponding organs in ancestral forms . . . Those [organs] might consequently be considered to have a kind of racial continuity that may be characterized as phylogenetic individuality." Danforth convincingly argues that fur hair, when compared with scales of fish and of reptiles, does not have that quality. And when comparing the pellage of different mammals (for all animals that on the lateral aspect of trunk between occipital region and tail and from the middorsal line laterally for $\frac{2}{3}$ of the distance to the midventral line) he finds no one-to-one homology between individual hairs: the bigger the animal, the larger the number of hairs on a comparable region of the body.

This is different for whiskers: their relative constancy in number points rather to their having the quality of phylogenetic individuality. The variation Danforth observed is

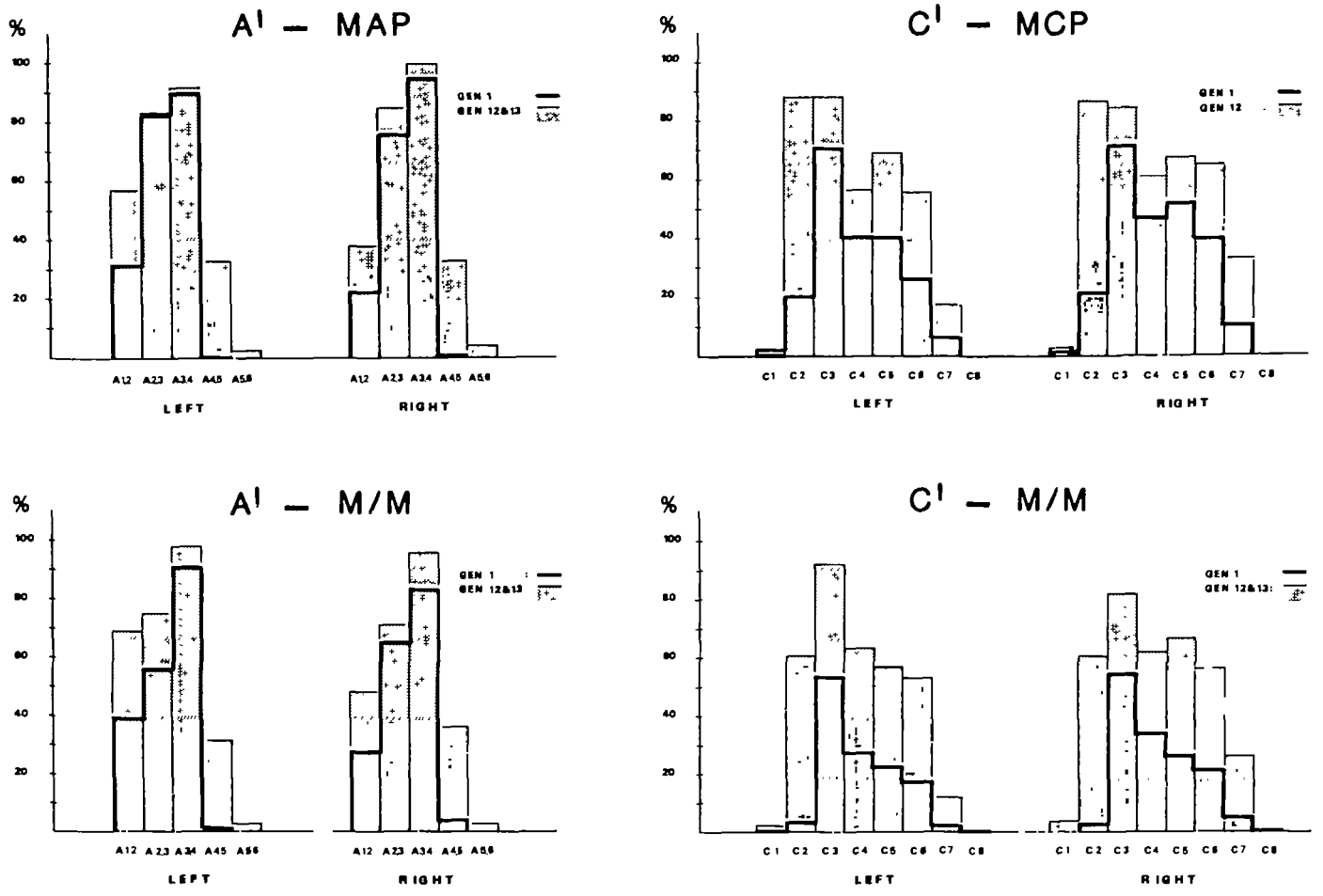


FIGURE 9 The frequencies by which sites of supernumerary of whiskers (SWs) within the two lines of fusion were occupied in three different strains. For each strain, the frequency of SWs at the left side are displayed to the left; for the right whiskerpad, to the right. 100% refers to all animals of a generation that possessed a SW at a given site. Top left pair of histograms gives the distribution of A'-whiskers in two generations of the MAP strain; bottom left pair, for same SWs but in two generations of the M/M strain. For the latter strain and for the same two generations, the distribution of C'-whiskers is given in right bottom pair of graphs. The right upper pair of graphs represents the C' distribution for two generations of the MCP strain. Sites of SWs are listed along the abcissa. For each strain, the first generation (GEN 1) is displayed by a solid black line; the more recent generation(s) is given by stippled zones. Number of animals per generation: for MAP, 180 in GEN 1, 132 in GEN 12+13; for MCP, 384 in GEN 1, 174 in GEN 12; for M/M, 186 in GEN 1, 136 in GEN 12+13.

modest and similar to that reported and reviewed by us[39]. This degree of variation does not interfere with Danforth's concept, but the very large variation we report in the present paper appears to do so. The distinction between having phylogenetic individuality or not, blurs in the light of the model proposed in the last section of the Discussion. In summary, the number of whiskers—skin appendages that are *early* to form—depends on the size of the skin area at the time they form; the number of hairs—skin appendages that are *late* to form—depends on the size of the skin area at the time the fur follicles are established.

The variability of whisker patterns in mice pertains not only to the mystacial, but also to the secondary vibrissae. Success in breeding for sublines with low and with standard numbers of the secondary vibrissae has been reported[9]. Grüneberg[12] observed in a congenitally hydrocephalic mouse strain, two, rather than the usual one, post-oral vibrissae. *All* post-oral vibrissae would have their onset as a pair, whose members fuse in the normal condition but remain separated in the mutant due to "skin strain" caused by the expanded skull. We have registered the tendency for certain strains to develop a supernumerary supraorbital (SO) vibrissa in addition to the normal two on each side. This condition enlarged the barrelfield: these whiskers, and also the infraorbital one, lie far away from the whiskerpad, but their barrels are adjacent to those of the mystacial vibrissae. This observation contributed to the notion that whisker sense is a special sense with its own cortical field[23,37]. SOs occurred most frequently in the M/M strain and we proved that it was possible to breed for them as well.

Interaction between the occurrence of supernumerary whiskers

In Results we report the frequencies of the various "contaminations" (see also the preceding discussion of MAP and MCP). For example, in AP/AP the B5 whisker disappeared over the generations, while A5, not considered as contamination, increased slightly. One cannot use these data to determine interaction of the occurrence of SWs (interdependence) because the frequencies with which several combinations occurred may well have been a by-product of breeding.

We approached this problem by determining the interdependence between SWs in the

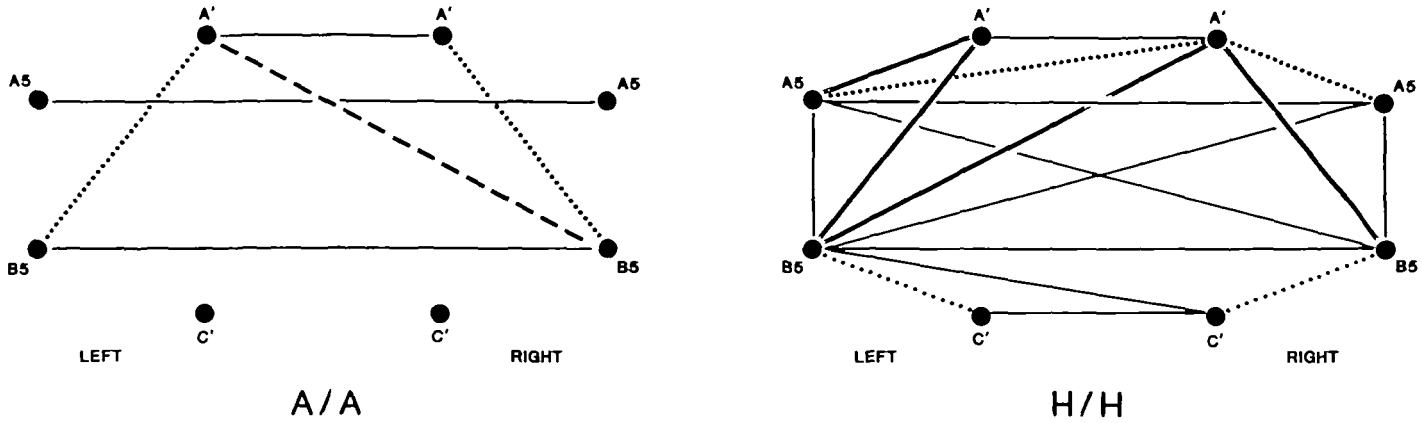


FIGURE 10 Summary of 28 tests of independence based on contingency tables, performed for the GEN A population of the A/A strain and for that of H/H. For each population, we tested all pairs that can be formed between whiskers A5, B5, A' and C' on both sides of the muzzle. Whiskers are represented by heavy dots. Open lines and dots indicate the existence of interaction between two whiskers, defined as "avoidance"; solid lines and solid dots, the existence of interaction defined as "attraction" (see text). Continuous lines indicate departure from independence at $P \leq 0.005$; broken lines, at $P \leq 0.025$; dotted lines, at $P \leq 0.05$. Where no lines exist, whiskers of the pair in question occur independently.

A generations of A/A and H/H. As described in Materials and Methods, these generations were formed by the offspring of parents that possessed the phenotype of the strains in question. We analyzed these offspring for they were close to the initial population, so that the "by-products" of breeding could be considered minimal. While the parental phenotypes were pure, the offspring shared many contaminations.

Our null hypothesis was that the presence of one element of each of the 28 possible pairs of types of SWs (on both sides) was independent of that of the other. The analysis was made separately for each strain. As in our previous study[39], expected values were obtained using contingency tables. Figure 10 presents the departures from independence showing the probabilities for rejection of the null hypothesis. Interdependence between two SWs may be of two kinds: 1) the occurrence of one SW of a pair *enhances* the chance for the second one to occur (positive interaction), or 2) two SWs *avoid* each other (negative interaction).

Positive interaction always occurred between the corresponding SWs across the midline in both strains (except for C's in the A/A strain), thus underlining the tendency towards symmetry for any particular character. In H/H, A5 and B5 interacted positively regardless of side, an interaction that did not exist in A/A.

In both strains A' interacted positively with its contralateral counterpart but not with any other SW. C' was independent in A/A, but interacted positively with B5 as well as with its contralateral counterpart in H/H.

Comparing the results pertaining to GEN A with those of a similar analysis performed on a group of animals that consisted of almost the entire initial population (Figure 5 in Van der Loos et al.[39]) we note 1) in GEN A both positive and negative interactions occurred while we now showed that the interactions in the initial population were only positive in nature; 2) for the phenotypes tested (A/A and H/H) only a few generations of selective breeding had led to two strikingly different patterns of interaction.

Lateral asymmetries

Lateral asymmetries of the phenotypes (Table I) for which selection was made occurred early in the breeding program. All phenotypes, except Do, showed a side preference: A5, A5+B5, and A' whiskers for the left side, and C's for the right. Preference for laterality of a particular type of SW does not differ between strains, e.g., C' occurred mostly on the right in CP/CP, MCP, AC/AC and M/M. In Table I, sidedness is expressed by ratios between the numbers of animals with left or with right asymmetries.

Between the first and last generation of the strains of classes I and II, the ratio shifted towards 1.00, with D/D as sole exception. In addition to this loss of side preference, the percentage of animals with an asymmetric distribution of elements constituting a given phenotype decreased except for AP/AP. Thus, for all the strains for which selection was successful (NOR, A/A, H/H, AAP/AAP, CP/CP, D/D), the percentage of asymmetric animals decreased. In the three strains that reached an improvement of almost 100 percent (NOR, A/A, and H/H), the left-right ratio became 1, and the percentage of asymmetrical animals exceedingly low. We take this as additional evidence for a high degree of homozygosity (see also fourth paragraph of Discussion). Conversely, the initial asymmetries in these strains, and the enduring asymmetries in the less successful strains, would be indicative of heterozygosity. Of course, this does not explain why under the condition of heterozygosity, SWs show side preference.

The proposed relationship between asymmetry and heterozygosity appears to be at odds with the generally held notion that greater asymmetry is the consequence of greater homozygosity of a population[5,32]. However, the authors who put forward this notion did not selectively breed for symmetry or for asymmetry, while we bred for symmetry. In a third article in this series we shall discuss bilateral asymmetry and side preference on the basis of results obtained through deliberate breeding for asymmetric distributions of SWs of given laterality (strains of class IV).

The strains of class III are less homogeneous with respect to the asymmetry in the distribution of SWs. Comparing the A's of M/M with those of MAP and its C's with those of MCP we note that for A's both strains are highly similar whereas for C's notable differences exist.

A model for the formation of the whisker pattern

We propose a model to explain the establishment of the various whisker patterns that we have described: we suggest that for all 13 strains a similar mechanism involving two sets of genes is responsible for the regular

arrays of whisker follicles, stereotyped within one strain but different between strains. A key assumption for our model is that zones of inhibition exist around each individual follicle primordium. Wigglesworth[46] and Schoute[28] made similar proposals to explain the distribution of the anlagen of bristles in insects and of leaves in plants, respectively (for general discussion see Meinhardt[20]). Figure 11*a* is a stylized rendition of the caudolateral part of a left whiskerpad. The model recognizes the fact that the development of the whisker rudiments begins with follicle δ (bottom right corner of figure; compare with Figure 12*c*; see also references 2, 41, 49, 51). The developmental "sweep" is towards rostral and medial (in the illustration towards the left and the top). Cells (or multicellular clusters) of the epidermal sheet are represented by hexagons. For our argument the distinction between cells and cell clusters is not important (we shall speak about "cells"). We postulate the release of a (humoral?) factor that, upon reaching the presumptive whiskerpad at its "δ corner," spreads in a rostral and medial direction. Each cell that responds to the factor becomes a center of proliferation leading to the formation of a follicle and is surrounded by an inhibitory zone within which cells are prevented from exhibiting that response. Assuming these zones to be of roughly equal size, each of them is indicated by a circle intersecting the cells that surround a "pre-follicular" cell. Thus, a next set of "follicle-generating" cells will be found at a given distance from the primordium of follicle δ. The "follicle-cells" are shown in different shades of grey; the darkest develops first. In parallel, the "inhibitory" circles are shown in different intensities. Inevitably, our illustration reflects a static situation. We chose the moment at which the travelling wave, just underway, had begun to contribute to the formation of the pattern. The mitotic figures in the top left corner of the cell-sheet symbolize the fact that, as the developmental sweep begins its travel, the future whiskerpad is still increasing its area, in particular at its rostro-medial end. The model implies that the number of follicles is a function of the total area of the presumptive whiskerpad, and that the "sweep" that turns uncommitted cells into either follicle stem cells or "banal skin" cells (determination), is limited in time: after it had passed over the region in question, subsequent cell divisions will lead to the formation of whisker follicles, and to an increase in area of the intervening skin, and not to the formation of yet more vibrissal follicles between those already laid down. A parallel may be drawn between the sweep proposed here and

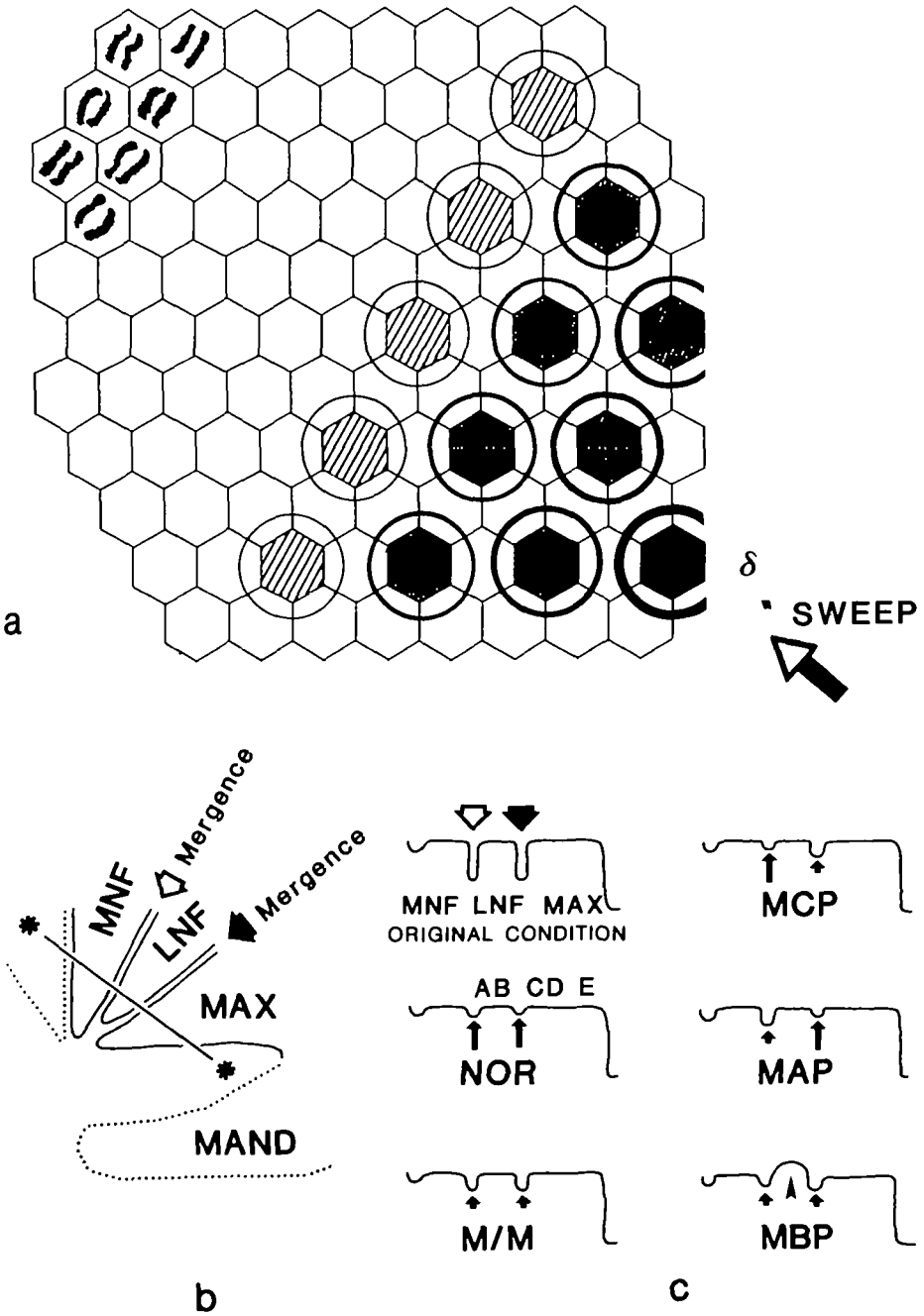


FIGURE 11 Drawings illustrating the proposed model for the formation of the whisker pattern. A stylized rendition of the caudolateral part of a (left) developing whiskerpad is given in *a*. In *b*, the left half of part of a mouse face is drawn in frontal view at a developmental stage in which the facial folds that constitute the muzzle are still separated by what will become the zones of mergence. The line between the two asterisks shows the orientation of the six sections normal to the epidermal surface illustrated in *c*. The hexagons in *a* represent cells (or sets of cells); the arrow indicates the direction of the "sweep," i.e., the developmental process that will lead to the determination of follicle stem cells. The stem cells are shown at different gray levels. The first one to be determined (that leading to the δ follicle) is darkest, the subsequent ones progressively lighter. The circles surrounding follicle-determined cells reflect "inhibitory zones" in which cells are prevented from becoming follicle stem cells. The thickness of these circles illustrates, again, the developmental gradient. Mitotic figures at upper left corner symbolize growth of whiskerpad. In *b*, lines of future mergence between medial nasal fold (*MNF*) and lateral nasal fold (*LNF*) and between the latter and the maxillary arch (*MAX*) are indicated by open and filled arrows, respectively. These processes are destined to form the whiskerpad; the mandibular process (*MAND*) is drawn in for clarity. In *c* the upper left drawing shows the situation before mergence. Arrows coded as in *b* point to lines of future mergence. The situation for the standard mice (NOR-strain) after complete mergence of the two lines of fusion (tall arrows) has ceased shows the disposition of the five rows of mystacial vibrissae (*A-E*). Reduced mergence (short arrows) in M/M animals permits supernumerary whiskers (SWs) to occur in zones corresponding to both lines of fusion. In MCP and MAP, only one of these two lines gives rise to extra elements. In MBP animals an SW occurs at a site that is not in direct relation to a zone of mergence, but to the crest of the LNF (arrowhead).

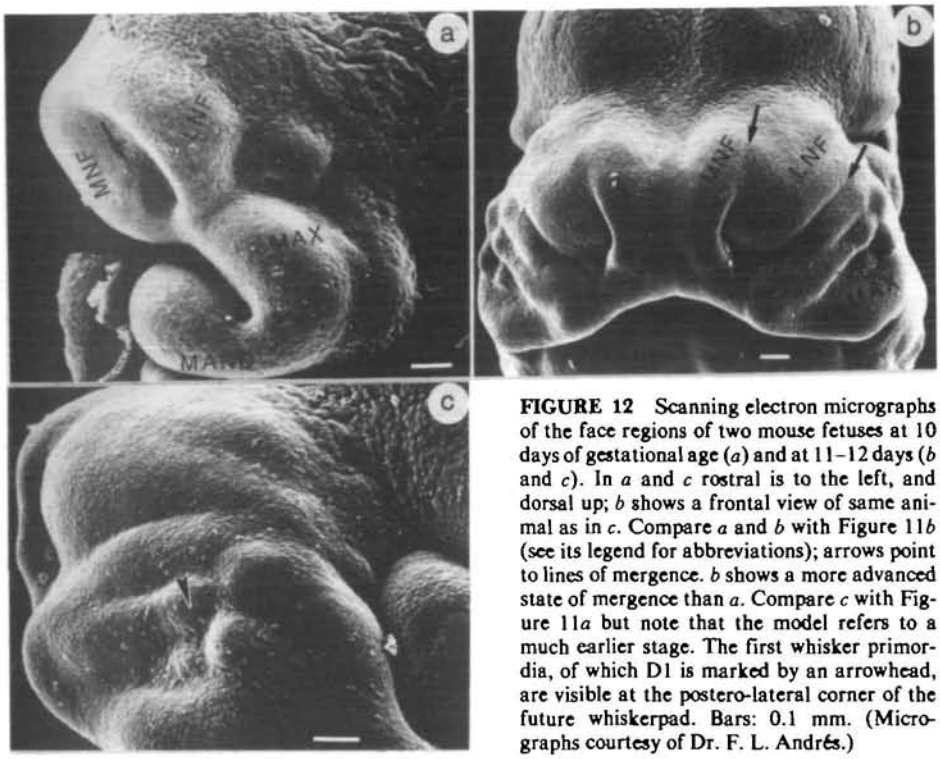


FIGURE 12 Scanning electron micrographs of the face regions of two mouse fetuses at 10 days of gestational age (*a*) and at 11–12 days (*b* and *c*). In *a* and *c* rostral is to the left, and dorsal up; *b* shows a frontal view of same animal as in *c*. Compare *a* and *b* with Figure 11*b* (see its legend for abbreviations); arrows point to lines of mergence. *b* shows a more advanced state of mergence than *a*. Compare *c* with Figure 11*a* but note that the model refers to a much earlier stage. The first whisker primordia, of which D1 is marked by an arrowhead, are visible at the postero-lateral corner of the future whiskerpad. Bars: 0.1 mm. (Micrographs courtesy of Dr. F. L. Andrés.)

the sequential formation of feather primordia of the chicken in vivo and in vitro[18].

Differences between mouse strains with respect to whisker pattern are based on differences in total whiskerpad area during the sweep. These differences then would lead to variations in number and pattern of whisker follicles. Figure 11*b* is a diagrammatic frontal view of a left mouse head during an early stage of development in which the three components of the muzzle, the medial and lateral nasal folds and the maxillary arch, are still discrete units. Figure 12*a* shows a scanning electron micrograph of the facial region at about the same age (see also Tamarin and Boyde[34]). The line segment connecting the two asterisks in Figure 11*b* shows the orientation of the six sections normal to the epidermal surface shown in Figure 11*c*. In reality, this line segment is a curve intersecting the three facial processes (Figure 12). The left top sketch of Figure 11*c* shows the presumed initial condition: the curve represents the epiderm that follows the contours of the three facial growth centers mentioned above. As argued earlier[37,39,40,51], supernumerary whiskers develop in the border regions, the zones of mergence[22,24,31] (fusion lines) between these centers. The essence of our hypothesis is that variation in mergence leads to areas of different size in one or in both of these zones and, thus, to the presence at those places of more or less SWs, or to their absence. In this view, less mergence signifies a larger local area, accompanied by an increased number of cells determined to constitute the vibrissal rudiments. The other drawings of Figure 11*c* illustrate the early conditions for different strains. The sizes of the arrows reflect the degree of mergence at the various sites. In one case, MBP, an enlargement of a part of the whiskerpad area that was not associated with a fusion line but located between rows A and B, i.e., on the crest of the lateral nasal fold, is postulated in order to explain the (relatively rare) occurrence of B' whiskers at that particular site.

An alternative explanation for the occurrence of more or less SWs within and outside the lines of fusion would be a variation in the size of the inhibitory zone surrounding each one of the whisker anlagen. While this mechanism, acting locally, may have contributed to the formation of B' whiskers and to that of SWs among the straddlers, we favor our first hypothesis for the majority of cases, partly on the basis of measurements of distances between follicles in adult animals of different strains, e.g., the distance between the B and C rows in MCP was larger than that in NOR (unpub. data).

We do favor the alternative hypothesis when we try to explain the Co,Do combination. This "artifactual" strain has been initiated with the question whether some whiskers are competitive, i.e., whether the presence of one would exclude another, particularly where limited space might prevent the simultaneous formation of two extra follicles. Until then, addition of whiskers had continued in certain regions (MAP, MCP, M/M), but we considered that potential crowding in the straddler row of whiskers could well provide an example of mutual exclusion. However, it turned out to be possible to create a new phenotype. The resultant crowding of follicles in the δ-corner of the whiskerpad made us believe that, here, a reduction in size of the inhibitory zones around the "follicle stem cells" may well have played a role.

While the developmental "sweep" that induces given cells to assume "follicle-quality," may be the expression of one set of genes (set *S*), another set (*M*) may selectively enlarge certain regions of the presumptive whiskerpad through more or less advanced mergence of the sulci between the nasal folds and between the lateral nasal fold and the maxillary arch or through pushing up the epiderm of the lateral nasal fold. It is the interaction between these two sets of genes that determines the final pattern of whisker follicles. Variations in the onset of expression of the *S* genes, and in the speed of the sweep that they encode, influence the area of the presumptive whiskerpad that, while in a sensitive state, confronts this developmental process. Variations in the expression of *M* genes locally increase the size of the sheet, thus making for more cells that are sensitive.

With respect to the lateral fusion line, our model may explain the occurrence of a CP/CP rather than a MCP pattern by: 1) a later arrival of the sweep at the lateral fusion line, i.e., when mergence is more advanced, or 2) an earlier mergence of the lateral fusion line. The fact that the most frequent C' follicle in MCP was the same as the one bilaterally occurring C' in CP/CP (i.e. C3') may give a lead to understanding the mechanism of mergence of the lateral fusion line. Similar reasoning can be applied when considering the MAP and the AP/AP strains whose most frequent SW is A3,4' (a whisker that lies on one arc with C3'). Returning to the lateral fusion line, it is interesting to note that there exist CP/CP animals whose only C' whisker is a C6'. Hence, an anterior site need not be taken up via the occupation of the more popular C3' site. In fact, it appeared to be possible to breed for C6's only.

Except for the NOR phenotype, the A5 follicle contributed most frequently to the other whisker patterns occurring in the initial population[39]. This may be associated with the fact that the sweep terminates at the rostral end of the A-row, where it is likely to arrive at times that vary between animals. It would have been this variation in timing that,

through inbreeding, became separated and thus led to two divergent groups of animals: NOR and A/A, the first strains to approach the 100 percent level of improvement.

In conclusion, we propose a model for the formation of the pattern of the follicles of the mystacial vibrissae, that aims at explaining the phenotypes of the different strains of mice that we successfully bred. The differences between the strains are genetically determined. We propose two sets of genes acting on the developing whiskerpad. Variations in time of expression of these genes (heterochrony[1,11]) would change the resulting phenotype, i.e., the pattern of sensory organs that calls forth their correspondingly modified representations in the brain.

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