

The Degree of Lateralization of Paw Usage (Handedness) in the Mouse Is Defined by Three Major Phenotypes

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Lateralization of paw usage in the laboratory mouse may be a useful model system in which to assess the genetic and developmental cause of asymmetry of hand usage. With a set number of paw reaches from a centrally placed food tube, individual mice from an inbred strain will exhibit a reliable number of left and right paw reaches. For a single inbred strain, there are approximately equal numbers of left-pawed and right-pawed mice, but strain differences have been reported in the degree of lateralization of paw preference. We reported a preliminary strain survey in which the strains appeared to fall into two groups of highly lateralized and weakly lateralized paw preference (Biddle *et al.*, 1993). We review here our expanded survey of genetically different strains and stocks of the laboratory mouse, including different species and subspecies. The major genetic trait is the degree of lateralization of paw preference and the strain differences appear to fall into three major classes of highly lateralized, weakly lateralized, and ambilateral preference. The trait exhibits both additivity and dominance in preliminary reciprocal crosses, depending on which strain pairs are used. The wide difference between strains that have highly lateralized and ambilateral paw preference suggests specific genetic tools that could be used to begin a genetic dissection of the causes of this trait. Preliminary assessment of the size of the corpus callosum in three strains with significantly different degrees of lateralization suggests that genetically determined deficiencies and absence of this structure are not the direct cause of the strain differences in the trait of degree of lateralization. In the expanded survey, some strains appear to exhibit a directional deviation from equal numbers of mice with left and right paw usage. Therefore, direction of paw usage may not be a genetically neutral trait, but replicate assessments and genetic tests are needed to confirm this.

KEY WORDS: Lateralization of paw preference; direction of paw usage; handedness; mouse strains; genetics.

INTRODUCTION

Left-right asymmetries of embryonic development continue to intrigue developmental biologists (Bock and Marsh, 1991). They are made more interesting because, in experimental genetic model systems like the laboratory mouse, the apparent left-right direction of visceral asymmetries can be

reversed or altered by single-gene mutations such as *situs inversus viscerum* (*iv*) on chromosome 12 (Layton, 1976; Brueckner *et al.*, 1989) and the transgenic insertional mutation “*inversion of embryonic turning*” (*inv*) on chromosome 4 (Yokoyama *et al.*, 1993). Other examples of alteration of left-right directional asymmetries have been reported, such as the visceral heterotaxias (anomalous placement of major organs and vessels) that appear to differ widely among common inbred strains of the laboratory mouse (Biddle *et al.*, 1991;

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Biddle and Eales, 1991), the reversal of the location of the posterior vena cava and hepatic portal veins that were reported in association with the *dominant hemimelia (Dh)* gene (Green, 1967) and the atriovisceral heterotaxias that occur in the NOD mouse strain as a diabetic embryopathy in response to overt maternal hyperglycemia but which are completely absent when the mother is normoglycemic (Morishima *et al.*, 1991). Nevertheless, the assessment of these directional asymmetries requires careful dissection and their genetic significance remains to be determined.

Lateralization of paw usage or handedness in the laboratory mouse may be another useful model system in which to assess the expression and biological causes of developmental asymmetries. Paw usage in a single-paw, food-reaching task is easy to assess and individual mice, when given a set number of paw reaches, demonstrate a highly reliable number of left-paw and right-paw reaches. Within an inbred strain, approximately half the mice are left pawed and half are right pawed. Among different inbred strains, the left-right direction of paw preference has appeared to be genetically neutral (Collins, 1968, 1969), but the degree of lateralization of paw preference is genetically determined because both highly lateralized (HI) and weakly lateralized (LO) strains were easily selected from a constructed heterogeneous stock (Collins, 1985).

In a survey of paw usage in 12 inbred strains, the degree of lateralization fell into two groups of highly lateralized and weakly lateralized paw preference, suggesting that a major gene may control some function and alternate alleles are expressed as weakly lateralized and highly lateralized paw preference (Biddle *et al.*, 1993). The hypothesis is made more plausible by considering that the highly lateralized and weakly lateralized groups of strains may be similar to the HI and LO phenotypes that were selected by Collins (1985). Also, in some strains, there was evidence of significant deviation of the numbers of mice to the left and right of equal paw usage that is independent of the degree of lateralization. Therefore, paw usage may be two separable traits of direction and degree of lateralization.

We have set a goal to assess a battery of strains for the foundation to our studies of genetically determined traits of visceral asymmetries. The paw usage traits are being added to this battery of strains. If the mouse model system is to be useful

for genetic studies, the range of possible phenotypes of paw usage needs to be documented for a wide sampling of genetically different and defined strains. We report here the continuation of this survey of the paw usage traits, including a review of our preliminary report (Biddle *et al.*, 1993). The major genetic trait continues to be the degree of lateralization of paw preference, but there now appear to be three major phenotypes of highly lateralized, weakly lateralized and ambilateral paw preference. The reciprocal F₁ generations between some of the strains were used to begin an assessment of what are the major genetic effects on these phenotypic differences. In the strain survey some strains show a directional deviation from equal numbers with left and right paw usage, suggesting that the direction of paw usage may not be a genetically neutral trait. Also, the strain survey has proved its preliminary usefulness by demonstrating there is no direct association between the degree of lateralization of paw preference and deficiencies or absence of the corpus callosum.

MATERIALS AND METHODS

The mice used in this study (Table I) are mostly inbred strains derived either from the laboratory mouse or from wild-caught mice of the *Mus musculus domesticus* species. SPRET-1 is an inbred strain derived from the separate species of *Mus spretus*. Some of the strains have been assessed and described previously (Biddle *et al.*, 1993) and they are listed and identified in Table I to facilitate comparisons. Except ICR/Bc, the inbred strains are maintained in our laboratory with continued inbreeding by sister-brother matings. We have not listed the specific generation of inbreeding in Table I but the strains are considerably beyond 20 generations. Details of these strains are available on request.

The newly assessed mice are described briefly as follows. CDS/Lay is an inbred strain that was derived by Dr. W. M. Layton (Dartmouth Medical School, Hanover, NH) by selection for response to the teratogen acetazolamide from a commercial random-bred stock of CD-1 mice. CD-1 (agouti) are mice from recently obtained CD-1 mice from commercial sources into which tyrosinase transgenes have been variably inserted and are expressed (Beermann *et al.*, 1991); they were a gift from Dr. S. Zackson (University of Calgary). CD-1 (random)

Table 1. Paw Usage Scores of the Inbred Strains and Stocks

Strain/stock	N	Right-paw entry (mean RPE \pm 95%)	Preferred-paw entry	
			Mean LPPE \pm 95%	PPE equivalent to LPPE
CDS/Lay	150	24.6 \pm 1.2	0.2441 \pm 0.0499	31.0
CD-1 (agouti)	16	25.3 \pm 4.4	0.2685 \pm 0.1763	31.6
PERU W-1-II	50	24.1 \pm 2.5	0.2945 \pm 0.0764	32.2
CD-1 (random)	100	23.9 \pm 1.7	0.3030 \pm 0.0489	32.4
PERU W-10-I	50	28.7 \pm 2.6	0.3735 \pm 0.1359	34.0
SWV ^a	150	27.2 \pm 1.9	0.4873 \pm 0.0644	36.4
I/LnJ	50	25.6 \pm 3.5	0.5104 \pm 0.1485	36.8
POSCH-2 ^a	50	22.0 \pm 3.5	0.5577 \pm 0.1510	37.7
ICR/Bc ^a	51	25.8 \pm 3.7	0.5597 \pm 0.1301	37.8
SIV/Lay	150	22.5 \pm 2.1	0.5690 \pm 0.0770	38.0
NOD/Lt ^a	150	20.8 \pm 2.1	0.6116 \pm 0.0858	38.7
CLA	50	25.0 \pm 3.8	0.6322 \pm 0.1821	39.1
PERU-W-10-II	50	27.4 \pm 4.2	0.6773 \pm 0.1293	39.8
DBA/2J	150	25.2 \pm 2.3	0.6807 \pm 0.1052	39.9
BALB/cByJ ^a	50	24.6 \pm 4.3	0.7325 \pm 0.1566	40.7
CBA/FaCam	150	21.9 \pm 2.5	0.7563 \pm 0.1101	41.4
SWR/J ^a	50	23.4 \pm 4.5	0.7887 \pm 0.1703	41.6
C3H/HeSn.Paf	50	28.6 \pm 4.7	0.8562 \pm 0.1533	42.5
“SPOTTED”	50	26.6 \pm 4.8	0.9560 \pm 0.2130	43.7
WB/ReJ ^a	50	22.1 \pm 5.0	0.9907 \pm 0.1849	44.1
WC/ReJ ^a	50	22.5 \pm 5.0	1.0047 \pm 0.1925	44.2
C57BL/6JLay.iv/iv	150	25.4 \pm 2.8	1.0309 \pm 0.1254	44.5
SPRET-1 ^a	50	26.8 \pm 5.1	1.0776 \pm 0.2328	44.9
C3H/HeHa.Pgk-1 ^{aa}	50	23.0 \pm 5.3	1.1324 \pm 0.2249	45.4
C57BL/6J.c ^{2j}	100	24.2 \pm 3.6	1.1530 \pm 0.1751	45.6
C57BL/6J ^a	150	20.3 \pm 3.0	1.2808 \pm 0.1246	46.6

^a Data from Biddle *et al.* (1993) with permission.

are discarded commercial CD-1 mice from the colony of Dr. Zackson. PERU W-1-II, PERU W-10-I, and PERU W-10-II are inbred strains of the PERU “Coppock” trapping of *M. m. domesticus* in Peru and bred by Dr. Margaret Wallace (Cambridge University, Cambridge, UK). I/LnJ were a gift from Dr. D. Wahlsten (University of Alberta, Edmonton, Canada) and were obtained originally from the Jackson Laboratory (Bar Harbor, ME). SIV/Lay is an inbred strain of mice that was selected by Dr. W. M. Layton from a random-bred stock that was homozygous for the *situs inversus viscerum* (*iv*) gene and originally obtained from the Jackson Laboratory. CLA is an inbred strain of wild-caught *M. m. domesticus* previously reported (Biddle and Nishioka, 1988). CBA/FaCam is an inbred strain obtain from Dr. M. Wallace (Cambridge University). C3H/HeSn.Paf is an inbred strain of C3H/HeSn and congenic for the *patchy fur* (*Paf*) mutation that was obtained from the Jackson Laboratory. “Spotted” is an inbred strain that is ho-

mozygous for a recurrence of the *belted* (*bt*) mutation that we derived from a random-bred stock of black mice that was maintained (but now discontinued) in our Animal Resources Unit and designated C57BL/HPB. C57BL/6JLay.iv/iv is an inbred strain of C57BL/6J that was constructed to be congenic for the *situs inversus viscerum* (*iv*) gene by Dr. W. M. Layton. C57BL/6J.c^{2j} is a coisogenic strain of C57BL/6J with a recurrence of the *albino* (*c*) mutation that was obtained from the Jackson Laboratory.

The mice were maintained as previously described (Biddle *et al.*, 1993). Reciprocal F₁ hybrids were produced between several strains, and in the abbreviation of the hybrids, the strain of the female parent is written first.

The mice were 8–10 weeks of age or older at the time of testing. They were fasted for 12–24 h and assessed in an unbiased test chamber (Collins, 1968) in which the food tube is equidistant from both sides. The test chamber accommodated up to

five mice in separate compartments. Either a flake food, called Snell's Love Mash at the Jackson Laboratory, or crumbled commercial diet was placed in the food tube. Mice were allowed 50 paw reaches for food and the number of right-paw entries (RPE) in 50 paw reaches was counted.

The measurements of paw usage that were established by Collins (1985) were used for consistency in these studies. The RPE score provides a measure of direction of paw usage. An RPE score of 0–24 indicates a left-pawed mouse, an RPE score of 26–50 indicates a right-pawed mouse, and a 25 RPE indicates an ambilateral mouse. A second measure, the preferred paw entry (or PPE), provides a measure of degree of lateralization of paw usage without regard to its left or right direction. The PPE score is derived from the individual RPE scores by the transformation $PPE = \text{absolute } |RPE - 25| + 25$ (Collins, 1985). For statistical comparisons, the PPE scores were transformed to logits where the logit of PPE (or LPPE) = $0.5 \ln(PPE + 1/6)/(50 - PPE + 1/6)$ (Collins, 1985; Tukey, 1977).

Conventional analytical methods were used to compare the direction of paw usage (RPE scores) and degree of lateralization (PPE scores) among the different strains and hybrids. The tests were taken from Sokal and Rohlf (1969) and are described when they are used in the Results. A procedure to differentiate cumulative distributions by numerical analysis was used to search for clusters or groupings of paw usage scores among the strains (Stewart, 1969; Stewart and Elston, 1973). The association between direction of paw usage (RPE) and degree of lateralization (PPE) among the strains was tested by a nonparametric test for association between continuous variables that is based on a contingency test (Elston and Stewart, 1970).

A preliminary assessment of the corpus callosum was made in three strains: C57BL/6J, SWV and CDS/Lay. Ten females and 10 males from each strain were anesthetized with tribromoethanol (Cunliffe-Beamer, 1983) and perfused with phosphate-buffered formalin by an intracardiac perfusion protocol (Dr. D. Wahlsten, University of Alberta, Edmonton, personal communication). The brains were removed and left in buffered formalin for at least 1 week. A free-hand, midsagittal razor section was made and the cut surface was stained with gold chloride (Schmued, 1990) to resolve the corpus cal-

losum using a protocol that was also provided by Dr. D. Wahlsten.

RESULTS

Strain Distribution Patterns of RPE and PPE Scores

The mean RPE and PPE scores for the 26 strains and stocks are listed in Table I. The strains that were assessed previously (Biddle *et al.*, 1993) are indicated in Table I. They are included here to facilitate comparisons. The PPE scores are listed as the means of the logit-transformed PPE (LPPE) scores from individual mice in each strain and the PPE equivalent to these mean LPPE scores are indicated. Similar to our previous findings, there was no significant difference between females and males in the new strains that were examined and the sexes are combined in Table I. The strains are rank ordered in Table I by increasing PPE scores in order to facilitate their analysis because the degree of lateralization of paw usage appears to be the major genetic trait.

The purpose of our continuing strain survey of paw usage is to document the major phenotypic differences among genetically defined strains within the mouse model system. Instead of conducting analyses of variance on the RPE and PPE scores and then assessing the significance of differences by, for example, least significant range tests, we used graphical analyses to explore the strain distributions of RPE and PPE scores for trends and groupings.

The rank-ordered strain distribution of RPE scores (from Table I) was explored by the graphical procedure of rankits (Sokal and Rholf, 1969). There appears to be no systematic deviation from one continuous and apparently normal distribution with a mean of approximately 25 RPE (Fig. 1a). When this continuous distribution of strain means of the RPE scores was numerically differentiated (Stewart, 1969; Stewart and Elston, 1973), there is a major peak at approximately 25 RPE (Fig. 1b), which suggests that most strains tend toward equal numbers of left-pawed and right-pawed mice. This numerical differentiation procedure uses the empirical data to assess the rate of change of a middle value (the midpoint) in a group of seven means relative to the three values to the left and to the

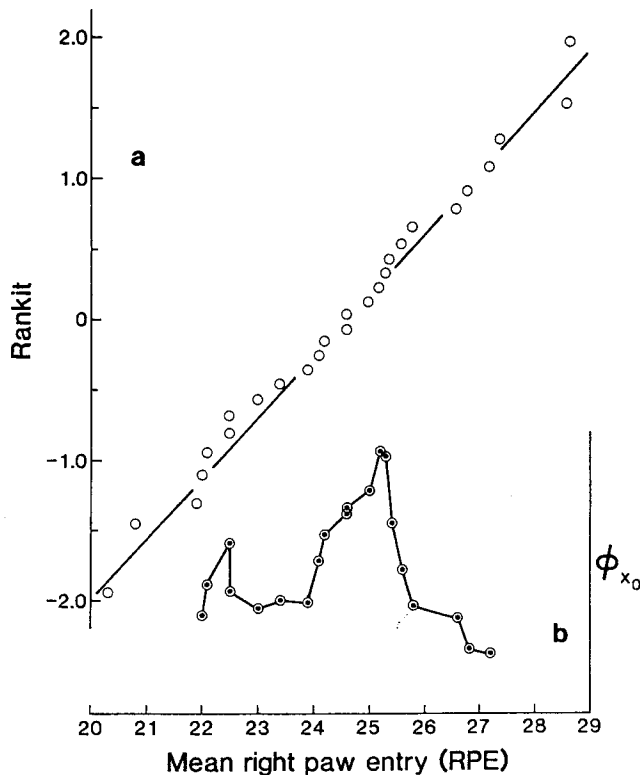


Fig. 1. Rank-ordered distribution of the mean right-paw entry (RPE) scores. (a) Strain means are plotted by the method of rankits (Sokal and Rohlf, 1969). The straight line, fitted "by eye," is the expected distribution if the RPE scores were sampled from one continuous and normal distribution. (b) Rank-ordered distribution is differentiated by the midpoint formula where the frequency at the midpoint is $\phi_{x_0} = 28/(-3x_{-3} - 2x_{-2} - x_{-1} + x_1 + 2x_2 + 3x_3)$ (Stewart, 1969; Stewart and Elston, 1973) and suggests that there may be a cluster of strains with left-paw deviation.

right (see formula in legend to Fig. 1b). There may be a minor cluster of strains at approximately 22.5 RPE, but whether these strains with a deviation toward left-paw usage have a genetically determined deviation needs to be tested in replicate assessments and genetic selection studies. So far, in the survey, only the NOD/Lt strain has been tested in replicate assessments and it shows a consistent deviation toward left-paw usage (data not shown and see Biddle *et al.*, 1993).

When the rank-ordered PPE scores (Table I), equivalent to the mean LPPE, were explored by the rankit procedure (Sokal and Rohlf, 1969), there is a systematic deviation from one continuous distribution (Fig. 2a). When this distribution was nu-

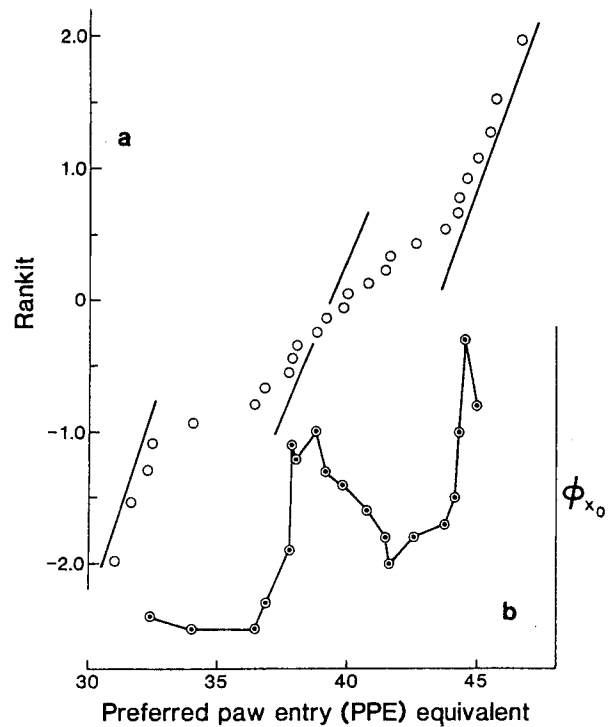


Fig. 2. Rank-ordered distribution of the mean preferred paw entry scores (PPE equivalent to the mean logit-transformed PPE scores). (a) Strain means are plotted by the method of rankits and the systematic deviation from one continuous distribution is emphasized by the three lines fitted "by eye." (b) Rank-ordered distribution is differentiated by the midpoint formula (see legend for Fig. 1) and suggests that there may be three clusters of PPE phenotype.

merically differentiated, there appear to be three clusters of highly lateralized, weakly lateralized, and ambilateral strains (Fig. 2b). Three clusters of phenotypes among genetically different strains suggest that there may be a major genetic effect controlling the degree of lateralization trait.

Test for Association Between RPE and PPE Scores

A question is whether there is any association among the strains between direction of paw usage (RPE score) and degree of lateralization (PPE score). The individual strain means of the RPE scores and the PPE equivalent scores do not exhibit any obvious linear correlation (Fig. 3a) when tested by the Spearman rank correlation test, $r_s = -0.196$,

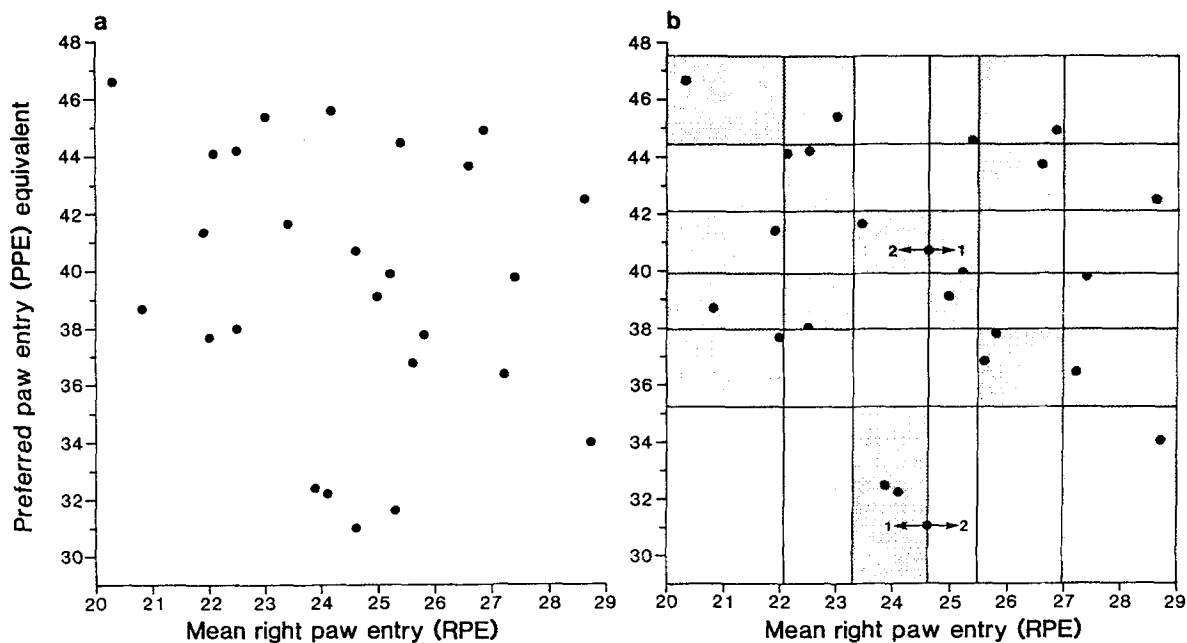


Fig. 3. (a) Comparison of the mean preferred paw-entry scores with the mean right-paw entry scores for the 26 strains and stocks. (b) The paired observations for 24 strains (see text) were used in the test for association (Elston and Stewart, 1970). A 6×6 grid is superimposed on the data and the cells containing paired observations are hatched. The arrows, labeled 1 and 2, indicate the order of the tests for the two strains with tied values for their RPE scores (see text).

and it is not significantly different from zero ($t = 0.979$, 24 df, $p > 0.10$) (Siegel, 1956).

If there is no linear correlation between the RPE and the PPE traits among the strains, there still might be an association or clustering of strains for the two traits. One of many tests for nonlinear association between two traits, when the underlying distributions are unknown, is a procedure that was developed by Elston and Stewart (1970) and that is based on a contingency table. The method is briefly outlined with the results because use of the test is not common. The equations to calculate the test statistic are given in the original publication (Elston and Stewart, 1970) or in published applications from this laboratory (Biddle and Fraser, 1979, 1986).

The requirements for the association test are that the number of paired observations is $N \times n$, where $N \times n$ is not prime, N and n are positive integers, $N > n > 1$, and $0.5 \leq n/N \leq 0.9$ or, preferably, ≤ 0.7 . Since there are 26 strains in the survey with paired scores of RPE and PPE, the requirements of the test are not met. A common procedure would be to discard strains by random sampling to reduce the number of paired observa-

tions to a number that would satisfy the test. In the present case, the CD-1 (agouti) and C57BL/6J. c^{2J} paired data (Table I) were discarded because of their close historical and, possibly, genetic relation to the CD-1 (random) and C57BL/6J strains, respectively. This reduced the number of paired observations to 24. Therefore, there are $N \times n = 6 \times 4 = 24$ paired observations and the requirements for use of the association test are met.

An $N \times N (= 6 \times 6)$ grid is superimposed on the 24 paired observations shown in Fig. 3b so that each column and each row of the contingency table contain the same number of observations ($n = 4$). The number of interfaces in an $N \times N$ grid is $2N(N - 1)$, and in this case, it is $2(6)(5) = 60$. The test statistic is the observed frequency of changing interfaces and is determined by counting in each column and in each row the number of times an interface changes from a cell with paired observations to a cell without paired observations, and vice versa.

Under the null hypothesis of no association between the mean RPE and the mean PPE scores of the different strains, the paired observations will be randomly distributed across the $N \times N$ grid. The

expected frequency of paired observations in each cell is the number of paired observations divided by the number of cells in the grid and is $Nn/NV = 24/60 = 0.4$, which is less than unity. If a paired observation falls in a cell, the observed number is greater than the expected number in that cell, whereas if no paired observation falls in a cell, the observed number is less than the expected number in that cell. A cell in the grid is considered positive if it contains observations and negative if it is empty.

The statistic is the number of times the sign changes (positive to negative and negative to positive) in passing from one cell to an adjacent cell in each column and in each row. In the present case of a 6×6 grid that contains four observations in each column and in each row, the expected probability of changing interfaces, with the null hypothesis of no association, is $p = 0.57$ and the standard error is 0.064 [see formulae in original publication (Elston and Stewart, 1970)]. In the test, the observed frequency of changing interfaces is compared with the expected probability (p) and its standard error.

In Fig. 3b, two strains, CDS/Lay and BALB/cByJ, are tied with RPE scores of 24.6. When they are ranked to the left and right of the line as shown by the direction of the arrows numbered 1 in Fig. 3b, the cells containing paired observations are shown as hatched. The number of interfaces in the 6×6 grid is $2(6)(5) = 60$, and 36 are changing from positive to negative, and vice versa. The observed frequency of changing interfaces is $36/60 = 0.60$ and is greater than the expected value of 0.57 under the null hypothesis of no association, but it is not significant compared with the standard error of 0.064. [When the ranking of the tied RPE scores of CDS/Lay and BALB/cByJ is reversed (direction of the arrows numbered 2 in Fig. 3b), the observed value of the test statistic increases to $37/60 = 0.62$, but again, it is not significantly different from expected.] Therefore, there is no evidence for an association between the RPE and PPE scores among the strains that have been tested.

Reciprocal Crosses Between Selected Strains to Assess Differences in PPE Scores

The major difference in paw usage among genetically different strains of the mouse appears to be in the degree of lateralization (PPE score). Our

previous study with a limited strain survey suggested that there were two major groups of highly lateralized and weakly lateralized paw preference and we used the C57BL/6J and SWV strains as prototypes of these groups (Biddle *et al.*, 1993) (see Fig. 4). With the expanded survey, a third or ambilateral group can be added and the CDS/Lay strain can be used to represent this group. The observed distributions of the RPE and PPE scores of the C57BL/6J, SWV, and CDS/Lay strains are compared in Fig. 4.

Reciprocal crosses were made between the C57BL/6J, SWV, and CDS/Lay strains in order to begin a genetic assessment of the PPE trait that is expressed by the parental strains (Table II). Reciprocal crosses were also made between the weakly lateralized DBA/2J and the highly lateralized C57BL/6J strains because a set of recombinant inbred (RI) strains (described by Bailey, 1981) has been constructed from this historical strain pair and it may be useful for the further genetic analysis of the paw preference trait. As found with the inbred parental strains, the RPE and PPE scores of females and males within the reciprocal F_1 hybrids were not significantly different and the sexes were combined for comparisons with the their parental strains. There were no differences between the reciprocal F_1 hybrids among the strain pairs in RPE scores and they do not differ from an average RPE value of 25 or equal paw usage (Table II). The mean LPPE scores of the four sets of reciprocal F_1 hybrids are shown as genetic diagrams in Fig. 5. There is no significant difference in LPPE scores between reciprocal F_1 hybrids among the four sets of strain crosses, which rules out potential maternal and X-linked effects, but only with these strain pairs. There is dominance deviation from additivity of the LPPE scores for the reciprocal F_1 hybrids from the CDS/Lay-SWV and CDS/Lay-C57BL/6J strain pairs that is in the direction of CDS/Lay, but the reciprocal F_1 hybrids from the SWV-C57BL/6J strain pair show additivity (from Biddle *et al.*, 1993). For the DBA/2J strain, which is weakly lateralized and phenotypically similar to SWV, the reciprocal F_1 hybrids from the DBA/2J-C57BL/6J strain pair shows dominance deviation in the direction of DBA/2J. Therefore, two strains, SWV and DBA/2J, with a similar phenotype of weakly lateralized paw preference exhibit a different phenotype in their respective F_1 hybrids with the highly lateralized C57BL/6J strain.

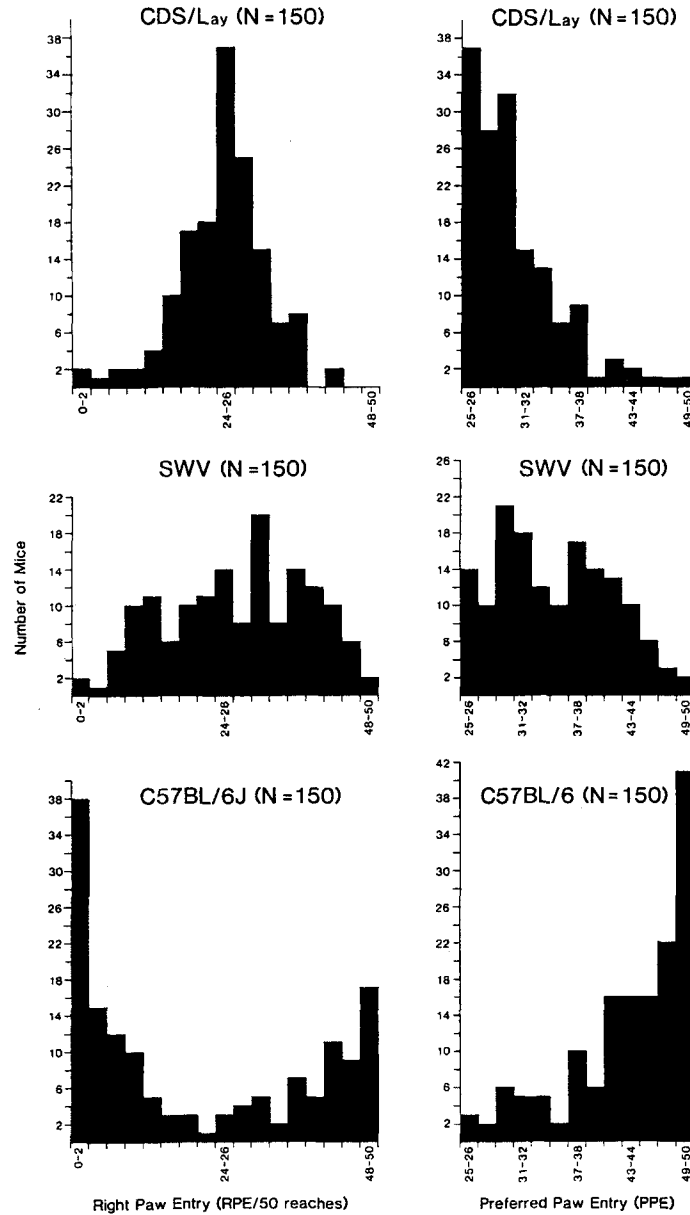


Fig. 4. Strain distribution of the right-paw entry scores compared with the preferred-paw entry scores for the C57BL/6J, SWV, and CDS/Lay strains to illustrate the phenotypic difference among the three major phenotypes of highly lateralized, weakly lateralized, and ambilateral paw preference, respectively. The data for C57BL/6J and SWV were illustrated previously (Biddle *et al.*, 1993).

To assess the additivity and dominance deviations suggested by the genetic diagrams (Fig. 5), the differences between the LPPE scores of the reciprocal F₁ hybrids and their respective parental strains were assessed by the *a posteriori* Student-Newman-Keuls test using a significance level of $\alpha = 0.01$ (Sokal and Rohlf, 1969) and the results are

shown in Table III. For the F₁ hybrids between CDS/Lay and SWV and between DBA/2J and C57BL/6J, dominance is complete and is in the direction of the more weakly lateralized parent. For the CDS/Lay and C57BL/6J strain pair, the reciprocal F₁ hybrids differ from both parental strains and the dominance deviation from additivity (Fig.

Table II. Paw Usage Scores of the Reciprocal F₁ Hybrids

F ₁ hybrid ^a	N	Preferred-paw entry	
		Right-pour entry (mean RPE ± 95%)	Mean LPPE ± 95% PPE equivalent to LPPE
CDS•SWV F ₁	150	24.3 ± 1.4	0.2888 ± 0.0452
SWV•CDS F ₁	150	25.4 ± 1.4	0.3151 ± 0.0464
CDS•B6 F ₁	150	25.4 ± 2.0	0.5511 ± 0.0841
B6•CDS F ₁	150	23.1 ± 1.9	0.4968 ± 0.0677
SWV•B6 F ₁ ^b	150	25.3 ± 2.8	1.0042 ± 0.1260
B6•SWV F ₁ ^b	150	23.0 ± 2.6	0.8946 ± 0.1159
D2•B6 F ₁	150	24.0 ± 2.5	0.8192 ± 0.1104
B6•D2 F ₁	150	26.5 ± 2.5	0.8376 ± 0.1150

^a Strain of female parent is written first in the abbreviations of the hybrids.

^b Data from Biddle *et al.* (1993) with permission.

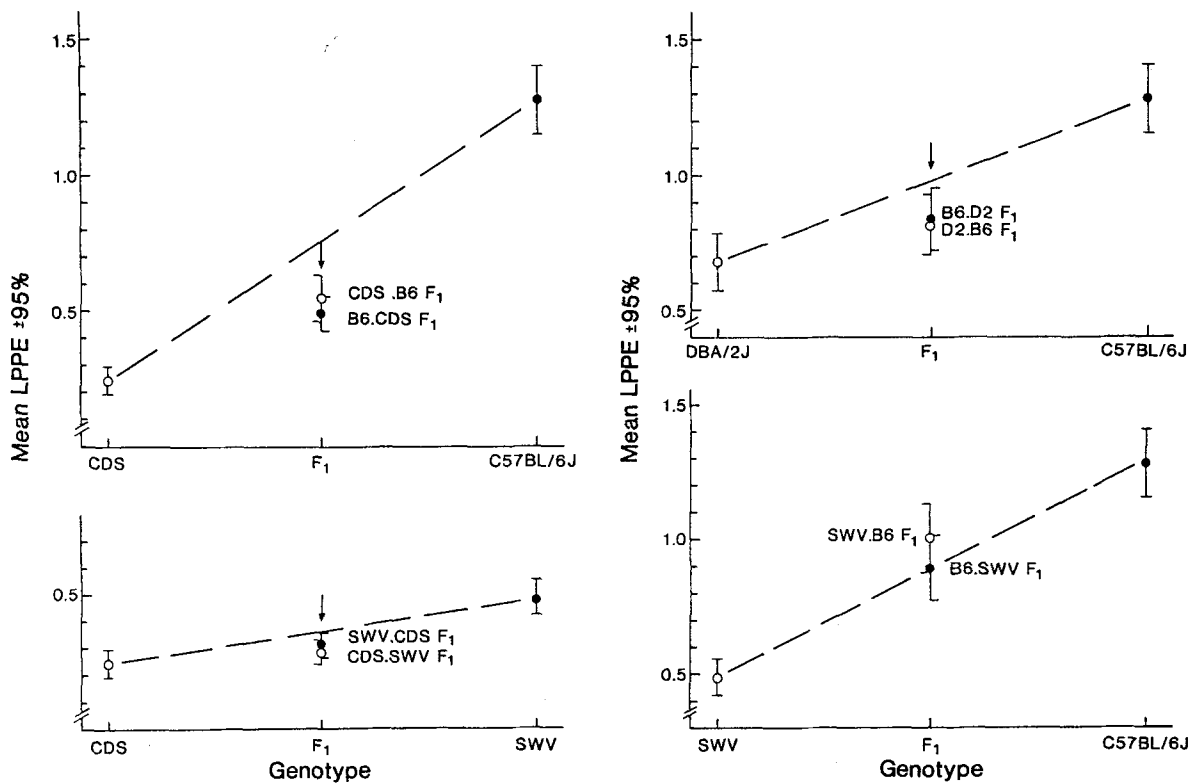


Fig. 5. Genetic diagrams of the means of the logit-transformed preferred paw entry scores (LPPE ± 95% limits) of the reciprocal crosses compared with their parental inbred strains. The expected means of the respective F₁ generations for an additive genetic model are predicted by the dashed line connecting the parental means. The arrows indicate the dominance deviations. The data for the SWV and C57BL/6J strains and their reciprocal crosses were illustrated previously (Biddle *et al.*, 1993).

5), toward the more weakly lateralized (that is ambilateral) CDS/Lay parent, is incomplete. For the SWV–C57BL/6J strain pair, the LPPE scores of the

F₁ hybrids show additivity. These observations of both dominance deviations and additivity of the LPPE scores of the reciprocal F₁ hybrids relative

Table III. Comparisons of the Degree of Lateralization (LPPE) Between the Reciprocal F_1 Hybrids and Their Respective Parental Strains Using the Student–Newman–Keuls Test^a

CDS	CDS•SWV F_1	SWV•CDS F_1	SWV
CDS	B6•CDS F_1	CDS•B6 F_1	C57BL/6J
SWV ^b	B6•SWV F_1	SWV•B6 F_1	C57BL/6J
DBA/2J	D2•B6 F_1	B6•D2 F_1	C57BL/6J

^a Strains and F_1 hybrids connected by a solid horizontal line are not significantly different by the SNK test at $\alpha = 0.01$.

^b SWV–C57BL/6 comparison is from Biddle *et al.* (1993) with permission.

to their parental strains suggest that there may be genetic heterogeneity in the cause of the phenotypic categories of ambilateral, weakly lateralized, and highly lateralized paw preference. At this early stage in the genetic analysis of the paw preference trait, these observations call for caution in making generalizations about the genetic control of the phenotypic trait in the species if genetic analysis is conducted with only one strain pair.

Association Tests Between Lateralization of Paw Preference and Albinism

At an early stage in the strain survey of paw usage behavior, there was an association between albinism (*c*, chromosome 7) and lateralization of paw preference. As the number of strains increased, the degree of lateralization (PPE scores) and the albino versus pigmented phenotypes segregated independently among the strains, and therefore, there is no direct or pleiotropic effect of the albino gene on lateralization of paw preference (Biddle *et al.*, 1993).

We also had the opportunity to test for potential associations between albinism and paw preference in two other ways. First, a coisogenic strain of the highly lateralized C57BL/6J was available from the Jackson Laboratory (Bar Harbor, ME). A mutation to albinism had occurred in the C57BL/6J strain and the substrain is called C57BL/6J.*c*^{2J}. It is maintained by mating a homozygous albino (*c/c*) mouse with a pigmented heterozygote (*c/+*) within the coisogenic strain. We tested both albino (*c/c*) and pigmented (*c/+*) genotypes that were bred in our laboratory (Table IV). There is no difference between females and males of the two genotypes

in RPE and PPE scores and the sexes are combined in Table IV. There is no difference between the paw preference scores from the two genotypes (Table IV) and from their parental C57BL/6J strain (Table I).

The assessment of the C57BL/6J.*c*^{2J} and the C57BL/6J strains is important for another genetic reason. The albino mutation of C57BL/6J.*c*^{2J} occurred at least 62 generations previously in the parental C57BL/6J strain at the Jackson Laboratory and our subline of C57BL/6J, listed as C57BL/6JBid, has been separated from the Jackson Laboratory strain by 60 generations of continued sister–brother inbreeding at the time we tested it. Therefore, considerable opportunity for subline divergence has elapsed between C57BL/6J.*c*^{2J} and our strain of C57BL/6J, but there has been no detectable change in phenotype of paw preference behavior.

The second way that association between albinism and paw preference was tested was with a heterogeneous group of genetically albino CD-1 mice that had a variable number of expressed copies of an inserted tyrosinase transgene (Beermann *et al.*, 1991) and, hence, were pigmented. These mice are listed in Table I as CD-1(agouti) and they can be compared to their parental stock of commercially obtained CD-1 mice, which are albino and listed in Table I as CD-1(random). There are no differences in paw usage scores between the pigmented and the albino CD-1 mice. Therefore, the tyrosinase gene (“albino” locus) has no effect on paw usage when randomly inserted into the ambilateral CD-1 background. This test was useful in another aspect. The CDS/Lay inbred strain was derived originally by selection for sensitivity to a specific teratogen from a commercial stock of CD-1 mice, beginning in 1973, and after 37 generations of selection, it has been maintained without selection by continued sister–brother inbreeding. Therefore, we can infer that the genetic determination of ambilateral paw preference has remained stable in these mice and was not changed by the genetic selection for sensitivity to teratogenic response and the subsequent inbreeding.

Assessment of Corpus Callosum

It has been suggested that weakly lateralized paw preference in some strains may be causally associated with deficiencies and/or absence of the corpus callosum (review by Lipp and Wahlsten,

Table IV. Paw Usage Scores of Albino (*c/c*) and Pigmented (*c/+*) Mice in the C57BL/6J•*c^{2J}* Coisogenic Strain

Genotype	N	Right-paw entry (mean RPE \pm 95%)	Preferred-paw entry	
			Mean LPPE \pm 95%	PPE equivalent to LPPE
<i>c/c</i>	50	26.1 \pm 5.1	1.0408 \pm 0.2183	44.6
<i>c/+</i>	50	22.4 \pm 5.3	1.2652 \pm 0.2768	46.5

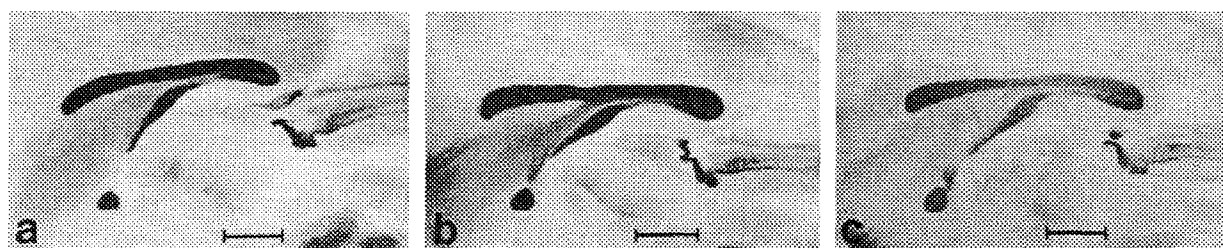


Fig. 6. Corpus callosum in the right face of a free-hand, midsagittal section of the formalin-fixed brain from a random adult female taken from the CDS/Lay (a), SWV (b), and C57BL/6J (c) strains. Gold chloride stain. Scale bar is 1 mm.

1992). This hypothesis was made more compelling by the report that the I/LnJ strain, which was known to lack completely a corpus callosum structure, is either weakly lateralized or ambilateral (Gruber *et al.*, 1991). The sample of I/LnJ mice that we assessed fell into the weakly lateralized group (Table I). The hypothesis has not been tested by a segregation analysis (or "test of transmission") to determine whether there is a genetic association between paw preference behavior and deficiency/absence of the corpus callosum. We examined the corpus callosum in a sample of 20 mice from each of the highly lateralized C57BL/6J, weakly lateralized SWV, and ambilateral CDS/Lay strains, and if the two traits are causally related, they should have cosegregated during development of the strains. All mice from the three strains had a corpus callosum (Fig. 6) within the range that has been defined as normal for the mouse (Wahlsten, 1982), and there was no evidence for deficiency or absence of the structure. Therefore, the strain survey demonstrates there is no direct cause and effect relationship between presence/absence of the corpus callosum and degree of lateralization of paw preference.

DISCUSSION

We began the survey of the paw usage trait among the historical and defined strains, which we

are assessing for developmental asymmetries, for two genetical reasons. First, a survey will begin to define what is the number of different phenotypes (the expression of the heritable differences) that are possible within the species and that are compatible with what is usually called "normal." Second, a survey will give some clues to the underlying genetic cause of the trait (Taylor, 1972). Potential allelic variation of genes, which may affect quantitative traits and which may have differed among the foundation stocks, will have assorted and become fixed during construction of the inbred strains. If there are two or a few distinct clusters of phenotypic classes of a trait among the inbred strains rather than a continuous distribution of differences, the trait may have a major genetic component rather than a multigenic cause. It is then the purpose of a genetic segregation analysis to distinguish between these modes of inheritance. When newer strains, which have been derived recently from other sources or wild populations, are added to this survey, they increase the power of the genetic inference of the survey. This strategy to identify genes involved in "normal" behavior has been discussed recently in detail (Festing, 1992). A third genetical reason can be added and that is potential functional association between traits will be suggested or rejected by the survey.

The genetically defined strains that are de-

scribed here should be viewed only as a very limited sample of the strains and stocks that are available for the mouse (Festing, 1979; Lyon and Searle, 1989; Potter, 1986). Nevertheless, the major difference among the strains in our sample is in the degree of lateralization of paw preference rather than in the direction of paw usage. There appear to be three major groups of strains with highly lateralized, weakly lateralized, and ambilateral paw preference (Fig. 2), and therefore, it is possible that one or a few major genes control the trait. With potentially a few genes controlling the degree of lateralization trait, an analysis of its cause, in terms of number of gene loci and their action, may be a practical exercise.

Since the phenotype of paw preference requires replicate assessment of individuals of the same genotype to assess it, the analysis of recombinant inbred (RI) strains would be the method of choice to pursue genetic assessment (Bailey, 1981). For the strains that differ in degree of lateralization in the present survey, only the highly lateralized C57BL/6J and weakly lateralized DBA/2J strains have been used to create a large set of RI strains. Since replicated assessment of individuals within a strain is necessary to assess quantitative traits, the use of the RI strain resources may be practical only at institutions that have already established breeding colonies of the RI strains because many RI strains are difficult to breed and the economics of large mouse colonies exceeds most single-investigator research grants.

An alternate approach to explore quantitative traits for their potential major genetic components has become feasible with the saturation of the mouse linkage map with PCR-assessed polymorphic microsatellite marker genes (Dietrich *et al.*, 1992, 1994; Copeland *et al.*, 1993). An F₂ generation between two different inbred strains can be assessed first for the quantitative trait and then systematically assessed for the three genotypes of the allelic differences at mapped marker genes across the genome. Tests for association and potential linkage of the quantitative trait are made with the homozygous marker genes. The advantages of this approach are that half of the F₂ individuals are informative and more complex genetic models with several marker gene loci can be systematically analyzed, with the only limitation being sample size. The main disadvantage is that the F₂ generation is

lost once it has been assessed and no further functional assessment of phenotype can be made on the individual mice.

An F₂ analysis between the weakly lateralized DBA/2J and the highly lateralized C57BL/6J strains may not be a practical system in which to begin a genetic assessment of the paw preference trait because their mean PPE scores are relatively close and there is considerable overlap between the parental distributions. Figure 7 is presented to illustrate this with the data from the parental DBA/2J and C57BL/6J strains and their reciprocal F₁ hybrids. If there was a difference at a single major gene locus between the two parental strains and if the heterozygous genotype, which is estimated by the F₁ distribution (Fig. 7b), could be removed by marker gene analysis, there would still be significant overlap between the two parental strains (Fig. 7a) that would be difficult to assess for association with homozygosity for the marker alleles. Also, more complex, multilocus models would be almost impossible to assess due to the very large sample size of F₂ individuals that would be required to subdivide the data.

The finding of an ambilateral phenotype for the degree of lateralization of paw preference in the present strain survey suggests that an F₂ analysis may be a practical method to begin a search for major gene components in the paw preference trait. For example, when the PPE scores of the ambilateral CDS/Lay strain are compared with the highly lateralized C57BL/6J strain, there is an approximately 15% overlap between the two strain distributions (Fig. 8a). The combined distributions of the PPE scores from the reciprocal F₁ hybrids between these two strains (Fig. 8b) shows complete overlap with the parental strains and dominance deviation of the mean toward the ambilateral CDS/Lay parent (see also Table III). If alternate alleles at a single major locus controlled the difference in degree of lateralization between CDS/Lay and C57BL/6J, the heterozygotes (measured by the F₁ generation in Fig. 8b) could be removed from the F₂ generation to see the parental CDS/Lay and C57BL/6J phenotypes and to provide evidence for segregation of the two alleles of the major gene. Also, the low frequency of overlap between the inbred CDS/Lay and C57BL/6J parents suggests that more complex, multilocus genetic models could be constructed and systematically assessed with a reasonable increase

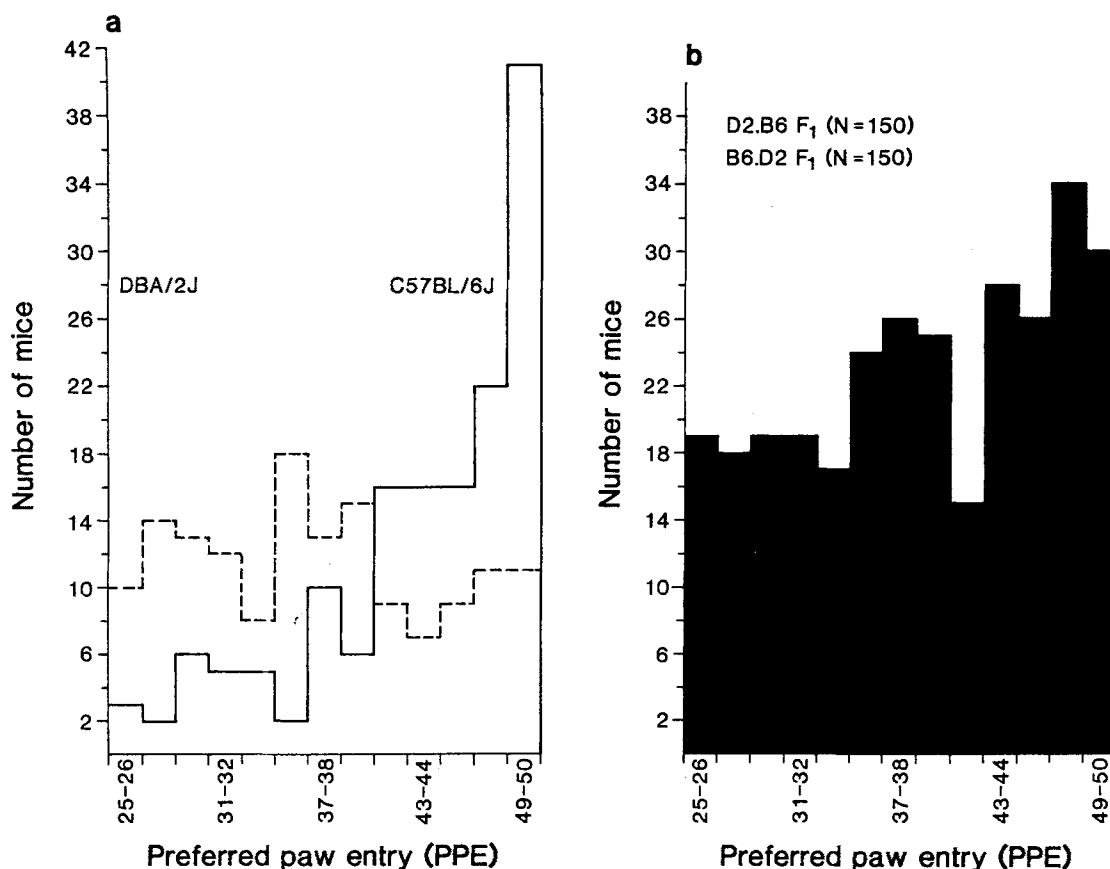


Fig. 7. Distribution of the preferred-paw entry scores from the inbred DBA/2J ($N = 150$) and C57BL/6J ($N = 150$) strains (a) compared with their combined reciprocal F_1 generations (b).

in the sample size of the F_2 generation. The rapidly assessed PCR-based microsatellite marker genes may provide the ability to do this.

The further assessment of the genetic cause of the degree of lateralization trait is simple to conceptualize, as in the above discussion, but it may be more complex in practice. Three major phenotypes of highly lateralized, weakly lateralized, and ambilateral paw preference from the strain survey serve only to suggest that a major genetic factor may control the trait. Preliminary assessments of the F_1 generations from reciprocal crosses between only a few strains from the major groups suggest the allelic differences between the strains that affect the trait do not all act in the same way. For example, the F_1 hybrids demonstrate that the difference between the weakly lateralized SWV and the highly lateralized C57BL/6J strains is additive but

the genetic difference between the similarly weakly lateralized DBA/2J strain and C57BL/6J exhibits complete dominance in the direction of the weakly lateralized DBA/2J strain (Fig. 5 and Table III). The only other reported assessment of paw preference in reciprocal crosses between strains with large sample sizes was between the selected HI (highly lateralized) and LO (weakly lateralized) strains (Collins *et al.*, 1993) and the difference appeared to be additive. Additivity, dominance deviations and complete dominance (Fig. 5 and Table III) for the differences between strains in degree of lateralization suggest that there may be different underlying genetic causes for the same behavioral phenotype.

A different method to assess lateral paw preference of the mouse was described recently (Waters and Denenberg, 1991). The apparatus, called a lat-

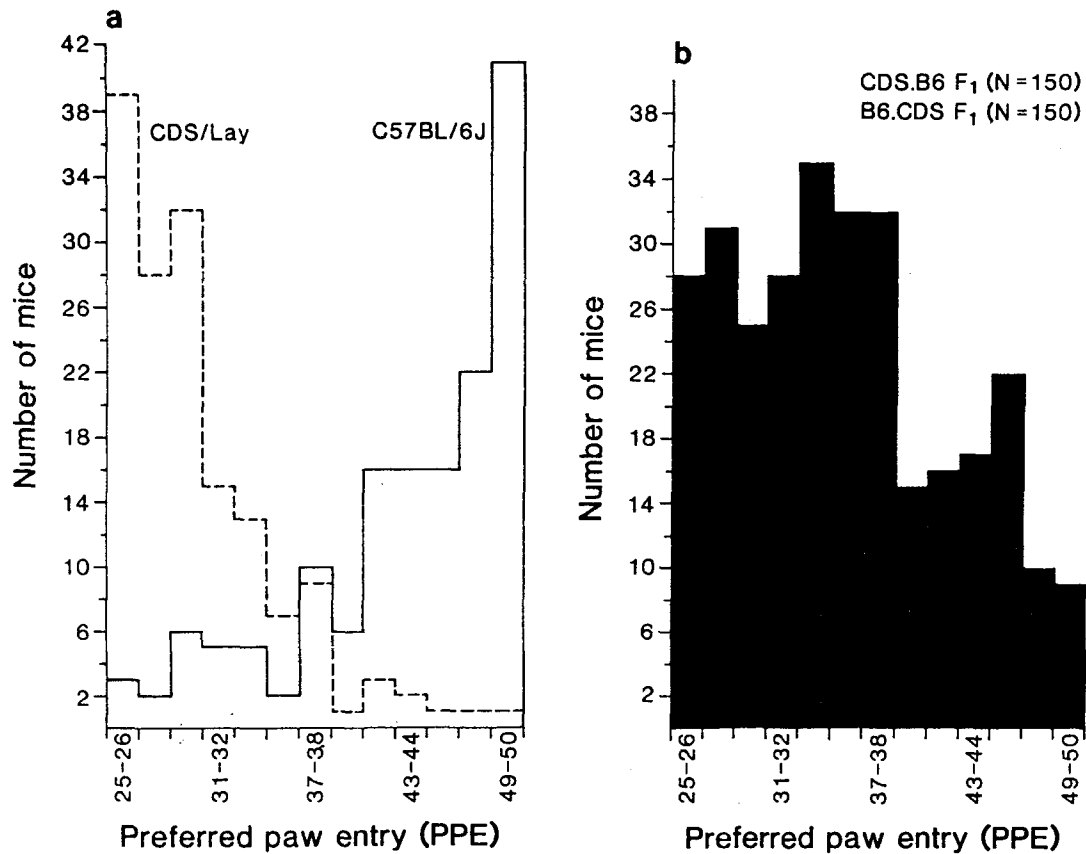


Fig. 8. Distribution of the preferred-paw entry scores from the inbred CDS/Lay ($N = 150$) and C57BL/6J ($N = 150$) strains (a) compared with their combined reciprocal F_1 generations (b).

eral paw preference (LPP) unit, is placed in the cage and the mouse is required to reach to the left or the right to remove flaked food from small chambers, which can be removed and weighed to assess the amount of food that was taken from the left and right chambers. The direction of lateralization and the degree of lateralization that are assessed with the LPP unit differed among a number of strains (Waters and Denenberg, 1994), but the differences in these measures were found to be independent of the paw preference measures of RPE and PPE that are assessed with the Collins test chamber. Support for a three-locus genetic model that determines the degree of lateralization that is assessed with the LPP test chamber was found with the set of NXSM (RI) recombinant inbred strains and demonstrated the utility of the RI strain technology to assess complex behavior. Additionally, the assessment of this same set of NXSM RI strains with the Collins test chamber found no genetic dif-

ferences and supported the conclusion that the two methods to assess paw preference are measuring two different behavioral/functional traits of the mouse. These results call for more concerted and, perhaps, systematic efforts to exploit the mouse behavioral model system.

Direction of paw usage in the Collins test chamber has been considered to be genetically neutral. Direction is said to be genetically neutral because the one and only attempt to select for it concluded that it did not respond to selection (Collins, 1969). The assessment was conducted within an existing highly inbred strain, but that important and pioneering study was done to demonstrate the stability of the paw usage phenotype within an inbred strain and to demonstrate that there was no residual heterozygosity for the trait that might be segregating in the inbred strains. Subsequently, the literature has assumed only that the direction of paw usage is genetically neutral. We found evi-

dence that some strains, such as NOD/Lt, may show directional deviation from equal numbers of left-pawed and right-pawed mice (Biddle *et al.*, 1993), and in the present report, several more strains may show a deviation toward left-paw usage (Fig. 1b). It will be important to conduct replicate assessments of these strains to determine the consistency of the deviation toward left-paw usage and to expand the survey to determine what are the extremes of the directional deviations that are possible in the mouse. Until large directional deviations from equal paw usage are found or selected from genetically segregating generations, it is premature to speculate further on these findings.

To date, there is no documented developmental variation that might plausibly lead to differences in the expression of degree of lateralization of paw preference, except for variation in the size of the corpus callosum. Genetically determined deficiencies and absence of the corpus callosum in the mouse may have behavioral consequences (review by Lipp and Wahlsten, 1992), and one hypothesis might be that deficiencies in or absence of this major commissural fiber tract between the left and the right cerebral hemispheres might cause an individual mouse to use its left and right paws equally or independently. The hypothesis was made compelling by the demonstration that the I/LnJ strain, which has a known total absence of the corpus callosum, is either weakly lateralized or ambilateral, particularly in young mice (Gruber *et al.*, 1991).

Preliminary genetic evidence suggested that there may not be a direct relationship between paw usage and size of the corpus callosum because second backcross progeny, from a cross of I/LnJ to the highly lateralized C57BL/6 strain, did not show a significant correlation (described by Lipp and Wahlsten, 1992). The wide strain survey appears to be of value in the debate about the role of the size of the corpus callosum and paw usage. We observed only normal corpora callosa in the sample of 20 mice from each of the ambilateral CDS/Lay, weakly lateralized SWV, and highly lateralized C57BL/6J strain and, which represent strains from the three major phenotypes of degree of lateralization. Therefore, there can be no direct cause and effect relationship between the size of the corpus callosum and the degree of lateralization of paw usage.

The neuroanatomical trait of deficiencies and absence of the corpus callosum that differs among

inbred strains appeared previously to be a genetically complex threshold trait of development. It is beginning now to be genetically dissected into its components with a clear three-locus model (Livy and Wahlsten, 1991). Also, the genetic components of the corpus callosum trait appear to be recovered quickly in a recently constructed set of recombinant inbred strains (Dr. D. Wahlsten, personal communication). This is providing both the impetus and the genetic tools to map the genetic determinants.

By comparison with the corpus callosum trait, genetic analysis of the behavioral traits of paw usage has only just begun. From our limited experience, there appear to be large phenotypic differences and, with newer tools for polymorphic marker genes, an opportunity to make significant progress toward genetic dissection of this task performance behavior in the mouse model system. What appears to be required is systematic work within a clear genetic framework.

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