J. Anat. (1990), **173**, pp. 87–95
With 4 figures
Printed in Great Britain

A quantitative study of cerebrovascular variation in inbred mice*

ROGER WARD, ROBERT L. COLLINS†, GILBERT TANGUAY AND DOM MICELI

Laboratoire de Neuropsychologie Expérimentale, Département de Psychologie, Université du Québec à Trois-Rivières, C.P. 500, *Trois-Rivières, QC, Canada G9A 5H7 and* † *The Jackson Laboratory, Bar Harbor, Maine* 04609, *USA*

(*Accepted* 15 *May* 1990)

INTRODUCTION

The circle of Willis (*circulus arteriosus cerebri*), that is formed on the base of the brain by the anastomoses of the internal carotid and basilar arteries, is a highly variable structure. Interspecific variation is amply discussed by Bugge (1974), but the question of individual variation within a single species remains somewhat of a problem. Variation of the human circle of Willis is described by Hasebe (1928) and by Alpers, Berry & Paddison (1959); it should be noted that while several authors (e.g. Brown, 1968*a*; Greene, 1935) credit Adachi (1928) with the systematic study of cerebrovascular variation in humans, it is clear from the text of Adachi's monograph that the work was carried out, and the relevant portion of the text written, by his colleague Kotondo Hasebe (cf. also Sakuma, 1961). The extent of variation is considerable, each of the 83 cadavers dissected by Hasebe showing a configuration of the arteries of the base of the brain that is recognisably different from each of the others.

Studies of cerebrovascular variation in nonhuman species (Sakuma, 1961: Brown, 1968*a*, *b*: Firbas, Sinzinger & Schlemmer, 1973) indicate that the range of variation is comparable to that found in man; indirect evidence suggests that some, at least, of this variation may be due to individual genotypic differences. Differences in the vascular architecture outside the circle of Willis, between inbred strains of rabbits, have been described (Edmonds & Sawin, 1936; McNutt & Sawin, 1943; Sawin & Nace, 1948; Sawin & Edmonds, 1949), as have differences in the configuration of the circle of Willis between A/Gr and C57BL/Gr mice (Froud, 1959). While the demonstration that inbred strains differ phenotypically is highly suggestive of an underlying genotypic difference, additional evidence is needed in order to specify the mode of inheritance. Such additional evidence is traditionally provided by breeding tests that are extremely time-consuming and require large numbers of animals. An alternative is provided by a technique developed in a slightly different context.

Morphometry of the lower jaw of the mouse can provide information about the phenotypic distance between inbred strains (see, e.g. Festing & Lovell, 1981, for a recent review); this information is generally consistent with the patchy historical record (Staats, 1966; Morse, 1978), in that strains known to be related by descent are more closely similar than strains known to be unrelated by descent, and generally consistent with estimates of genotypic distance based on the strain distribution of

* This article is dedicated to Professor Roger Saban, Laboratoire d'Anatomie Comparée, Muséum National d'Histoire Naturelle, Paris, on the occasion of his retirement.

biochemical and immunological polymorphisms (Taylor, 1972; Ward, 1985). In particular, the family of C57 strains, derived from Miss Lathrop's stocks of fancy mice in the 1920s, stand apart from other inbred strains. The purpose of the observations described below was therefore twofold: to compare the individual variation within a genetically defined but heterogeneous sample with that previously described in genetically undefined rodents (Brown, 1968*a*; Firbas *et al.* 1973), and to enquire whether the variation between inbred strains is amenable to quantitative analysis similar to that which has been brought to bear on the morphology of the murine mandible (Festing & Lovell, 1981).

MATERIALS AND METHODS

Mice from a genetically defined, heterogeneous stock (a control line from a selective breeding experiment – Collins, 1985), and from three inbred strains, were studied. The heterogeneous stock was derived from the eight-way cross between *Mus musculus molossinus*, *Mus musculus castaneus*, and the inbred strains BALB/cJ, C57BL/6J, DBA/2J, LP/J, RF/J and SM/J, and had been maintained for 8 generations by random mating when the individuals were taken for study. The inbred mice were the offspring of breeding pairs of C57BL/6J or 129/J mice obtained from the Jackson Laboratory, or the descendants of breeding pairs of BALB/cCF mice kindly provided by D. Wahlsten of the University of Waterloo; in view of the unfortunate history of contamination of some commercial stocks of 'BALB/c' mice (Kahan, Auerbach, Alter & Bach, 1982), Wahlsten (personal communication) has recently examined his existing BALB/cWah substrains, derived from the same initial population of BALB/cCF mice as those whose descendants were used in the present study, and found them to be homozygous for the alleles *Pep-3*^{a}, *Pgm-1*^{a}, *Gpi-1*^{l}, *Hbb*^{d}, *Es-3*^{a}, *Es-10*^{a}, and the wild-type allele at the *rd* locus. It is therefore unlikely that the sample of BALB/cCF mice used in this study was contaminated.

Mice between four and six weeks of age were deeply anaesthetised by an intraperitoneal injection of sodium pentobarbitone (60–70 mg/kg) and perfused through the heart with isotonic (10·7 g/litre) sodium nitrite followed by 10% buffered formalin and finally by 5–10 ml of India ink (Pelikan). Each mouse was wrapped in paper moistened with neutral buffered 10% formalin and stored overnight at 4 °C; the brain was removed, and finally the dura mater was removed by dissection under fresh 10% formalin. The arteries of the base of the brain were traced at a final magnification of $\times 18$ by means of a drawing attachment fitted to a Wild M650 dissecting microscope.

Since the preparations obtained from the genetically heterogeneous mice were made over a relatively long period of time and since the preparations were stored in formalin, a quantitative investigation of this material was not carried out because such data would be heavily contaminated by postfixation swelling and distortion. Quantitative analysis of the inbred strains proceeded as follows. Each preparation was examined within 24 hours of the removal of the brain. The Cartesian coordinates of each of the landmarks indicated in Figure 1 were determined, to the nearest millimetre at the scale of the camera lucida tracings, by means of a Numonics 2000 graphics tablet attached to a microcomputer. In order to orientate each tracing, the terminal bifurcation of the right internal carotid artery (Point 1 of Figure 1) was taken as the origin ($x = 0$, $y = 0$) and that of the left internal carotid was subject to the constraint that $y = 0$. The set of 45 Euclidian distances

$$D_{jk} = [(x_j - x_k)^2 + (y_j - y_k)^2]^{0.5}, \quad \text{where} \quad j < k,$$

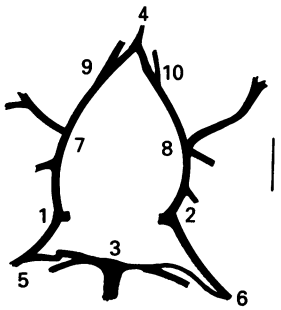

Fig. 1. Camera lucida tracing of the circle of Willis of a C57BL/6J mouse after perfusion with India ink. The landmarks are: 1, right and 2, left internal carotid arteries; 3, termination of the basilar artery; 4, junction of the anterior communicating arteries; 5, right and 6, left junctions of the posterior cerebral and posterior communicating arteries; 7, right and 8, left middle cerebral arteries; 9, right and 10, left anterior cerebral arteries. Scale bar, 1 mm.

between all pairs of landmarks was computed for each specimen. In order to eliminate the possible effect of an overall 'size' factor, these distances were then converted to standard scores for each inbred strain

$$Dz_{jk} = (D_{jk} - \mu D)/\sigma D,$$

where μD and σD stand for the mean value and standard deviation of the distribution of values of D for the inbred strain in question.

Analyses of variance of distances were carried out to determine which of these differed significantly between the three inbred strains; since 45 such comparisons were carried out simultaneously, in order to reduce the Type I error rate the criterion of significance was set to the Bonferroni probability, $0{\cdot}05/45 = 0{\cdot}0011$ (e.g. Bailey, 1985, 1986). In order to enquire whether the significant differences might be ascribable to additive genetic variation, the analysis proceeded as follows.

One of us (Ward, 1985) has previously argued that if phenotypic variation can be adequately described by a simple additive genetic model, then the magnitude of a phenotypic difference between two members of a set of inbred strains will be linearly related to the degree of genetic dissimilarity between them; or, alternatively, the magnitude of the phenotypic difference will be inversely related to the degree of genetic similarity. A simple measure of the degree of genetic dissimilarity is provided by Roderick's (1980) compilation of enzymatic and immunological polymorphisms known at 155 loci in the 44 inbred strains of mice maintained by the Jackson Laboratory; from this compilation, the proportion of loci at which different alleles are fixed in any two inbred strains may be determined. Analysis of the 44×43 triangular matrix of all possible values of these proportions (Ward, 1985) shows that while the first extracted eigenvalue accounts for 59·3% of the explained variance, the second and subsequent values each add less than 8% to the proportion of explained variance, and thus that the matrix is adequately described by a simple univariate measure of genetic dissimilarity. It is evident that if a proportion p of polymorphic loci carry different alleles, and p is taken as a measure of genetic dissimilarity, then the proportion $q = (1-p)$ may be taken as a measure of the degree of genetic similarity.

A measure of the overall phenotypic difference between two inbred strains may be derived, in the following way, by the decomposition of the mean squares obtained in an analysis of variance. The difference between two inbred strains may be thought of as a fixed treatment effect (Sokal & Rohlf, 1981), and the between-group mean square

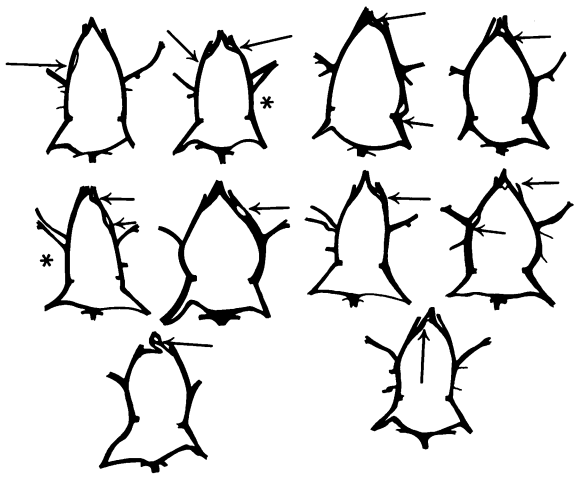

Fig. 2. Preparations of the circles of Willis of ten genetically defined, heterogeneous mice. Note that each specimen shows at least one 'buttonhole' anomaly (arrows), two showing in addition either a biradicular origin or reduplication of the middle cerebral artery (asterisks).

is thus an unbiased estimate of the sum of the variance ascribable to experimental error and a quantity equal to n times the added variance component due to strain differences, where n is the number of within-cell replications. The variance ascribable to experimental error is estimated by the within-group mean square, and the arithmetic necessary to estimate the added variance component is thus straightforward. For those comparisons in which an initial analysis revealed significant differences among the three strains, paired comparisons were carried out in order to estimate the added variance component, and these were summed to provide an overall estimate of the phenotypic difference between the two inbred strains in question.

RESULTS

The results presented below were obtained from ten mice of the genetically heterogeneous stock and ten male mice of each of the three inbred strains.

Qualitative

The circle of Willis in the mouse forms a polygonal network of arteries on the basal surface of the brain (Fig. 1). It is supplied by the paired internal carotid arteries and the basilar artery, and from it arise the paired anterior, middle and posterior cerebral arteries together with various numbers of small branches, and the unpaired olfactory artery.

The basilar artery, lying in the midline of the basal surface of the brainstem and arising from the fusion of the vertebral arteries, terminates in a quadrifurcation to give rise to the paired anterior cerebellar arteries and the *partes basilaris* of the posterior communicating arteries. These latter arteries pass laterally between the surface of the brainstem and the posteroventral face of the cerebral hemisphere to join with the *partes carotica* of the posterior communicating arteries to form the two posterior cerebral arteries. The *pars carotica* of the posterior communicating artery is one of two equal-sized terminal branches of the internal carotid artery, which divides on the base of the cerebral hemisphere slightly caudal to the hypothalamus; the *pars carotica* passes caudally and laterally to join its *pars basilaris*. The second branch, the anterior

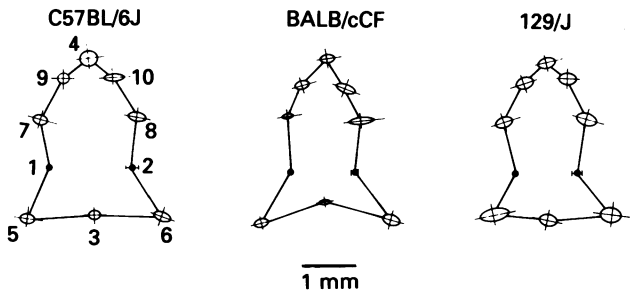

Fig. 3. Schematic representation of the results of measurement of the circle of Willis in three inbred strains of mice. The points numbered 1–10 correspond to the landmarks of Figure 1. Point 1 is taken as the origin and its coordinates are invariant; Point 2 is constrained to vary only with respect to its X-coordinate. The bivariate means of the remaining points are indicated together with their 95% confidence limits.

Table 1. *Added variance components, expressed as percentages of the explained variance, responsible for significant differences in distances between landmarks of the circle of Willis.*

	From point								
To point	1	2	3	4	5	6	7	8	9
	(a) Differences between C57BL/6J and BALB/cCF mice								
2	68%	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—
4	39%	—	55%	—	—	—	—	—	—
5	51%	—	—	49%	—	—	—	—	—
6	33%	—	—	—	—	—	—	—	—
7	69%	62%	—	—	—	—	—	—	—
8	43%	—	—	—	—	—	70%	—	—
9	—	44%	—	68%	—	—	53%	50%	—
10	34%	—	—	61%	—	—	65%	—	—
	(b) Differences between C57BL/6J and 129/J mice								
2	65%	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—
5	—	30%	—	—	—	—	—	—	—
6	48%	—	—	38%	—	—	—	—	—
7	42%	30%	—	—	—	—	—	—	—
8	26%	32%	46%	—	—	29%	—	—	—
9	—	—	33%	—	—	—	—	—	—
10	—	49%	67%	—	—	—	—	—	—
	(c) Differences between BALB/cCF mice and 129/J mice								
2	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—
6	—	—	—	—	34%	—	—	—	—
7	—	53%	—	—	35%	—	—	—	—
8	—	—	—	—	—	—	69%	—	—
9	—	42%	—	62%	—	—	46%	—	—
10	—	56%	59%	51%	—	—	72%	—	—

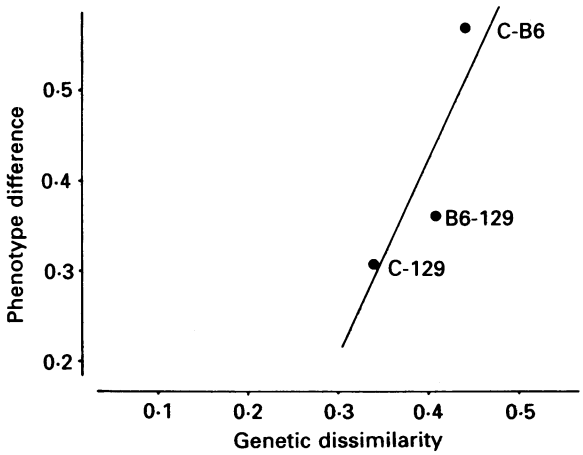

Fig. 4. The relationship between the degree of phenotypic difference as defined in the text, and the degree of genetic dissimilarity calculated from Roderick's (1980) data, for different pairs of the three inbred strains C57BL/6J (B6), BALB/cCF (C), and 129/J (129).

communicating artery, passes rostrally, curving round the lateral margins of the hypothalamus, giving off as major branches the middle and anterior cerebral arteries, to eventually join with its contralateral homologue beneath the anterior margin of the optic chiasma; from this point of fusion arises the olfactory artery. In the majority of specimens the chorioidal artery could be seen to arise caudally to the middle cerebral artery, more variable lenticulostriate arteries arising from the same region of the circle. Camera lucida tracings of the circles of Willis of the ten genetically heterogeneous mice are shown in Figure 2. The most striking finding was that each specimen showed at least one of the anomalous features described by Brown (1968*a*) in the rat; one specimen showed a biradicular origin of the right middle cerebral artery, two showed reduplication of one of the middle cerebral arteries, and each of the ten specimens showed at least one configuration of collateral branches, described by Brown (1968*a*) as 'buttonholes', in which the anterior communicating artery divides and then rejoins. No such anomalous feature was seen in any of the 30 inbred mice examined, and the difference between heterogeneous and inbred mice is strikingly significant ($P = 1{\cdot}18 \times 10^{-9}$, Fisher's exact test).

Quantitative

The results of the measurement of 10 specimens of each of the three inbred strains are shown in Figure 3. The terminal bifurcation of the right internal carotid was taken as the origin ($x = 0$, $y = 0$) for each specimen and is hence invariant, and the contralatral homologue was constrained to vary only with respect to its X-coordinate. The positions of the eight remaining landmarks are indicated as the bivariate means and 95% confidence limits (Sokal & Rohlf, 1981) of the X- and Y-coordinates. Inspection suggests that a marked difference exists between C57BL/6J mice on the one hand and the two remaining strains on the other; the circle of Willis of C57BL/6J mice is wider but of comparable rostrocaudal length. This initial impression is borne out by the results of the analyses. The variance components estimated by the second series of analyses are tabulated as Tables 1*a*–1*c*. The relationship between the overall measure of phenotypic difference between pairs of strains and the genotypic dissimilarity between them is indicated in Figure 4. A simple linear relationship accounts for 72%

of the variance and thus gives a reasonably good account of the data, consistent with the notion that interstrain differences in the configuration of the circle of Willis are due to additive genetic differences.

DISCUSSION

The most striking feature of our results is the marked difference between genetically heterogeneous and inbred mice. Each of the 10 heterogeneous mice showed at least one anomalous feature, whereas none of the 30 inbred mice did so. These anomalies are similar to those described in the genetically undefined rat by Brown (1968*a*), in particular the 'buttonholes' in the anterior portion of the circle of Willis, and presumably arise by similar unknown developmental mechanisms. Our finding that genetically heterogeneous mice are qualitatively different from inbred mice agrees with the previous finding (Festing, 1976) that the morphology of the mandible is more variable in genetically undefined 'Swiss' mice and in F2 hybrids than in genetically homogeneous F1 and inbred strains.

In a comprehensive study of arterial variation in three inbred strains of mice, Froud (1959) found that the *pars basilaris* of the posterior communicating artery was unilaterally or bilaterally absent in 10 of 11 C57BL/Gr mice and was reduced in size in the remaining specimen. We found no such anomaly in any of the 10 C57BL/6J mice we examined. It is unlikely that the total absence of an artery in one set of material and the presence of this artery in our material is due to technical differences between the two studies; we suggest that divergence between the two substrains (e.g. Ward, 1985) may be responsible for this discrepancy. Froud (1959) also reported that for the 'qualitatively most striking' variations of arteries other than those of the circle of Willis, C57BL/Gr mice were considerably different from A/Gr and CBA/Gr mice. Later work has shown that the set of C57 strains in general, derived from Miss Lathrop's stock of fancy mice in the 1920s (Staats, 1966: Morse, 1978), can be distinguished from other inbred strains either on the basis of immunological and biochemical polymorphisms (Taylor, 1972) or on the basis of morphometric analysis of the mandible (Festing & Lovell, 1981), and our own results support this distinction.

The intensity of the linear relationship between phenotypic differences and genetic dissimilarity is fairly pronounced (Fig. 4). It thus appears (Ward, 1985) that the heritability, estimated by conventional biometric techniques, of cerebrovascular variation is likely to be quite high. Falconer (1981) has pointed out that, counterintuitively, traits with high heritabilities are in general biologically less important than those with low heritabilities. This paradox comes from the fact that biologically important traits are subject to more intense selection pressure than are less important or neutral traits and the additive genetic variance underlying an initially high heritability is eliminated by natural selection. The apparently high heritability of cerebrovascular variation in mice may thus be interpreted in one of two ways. It may be the case that this variation is unrelated to fitness; or it may be the case that the consequences of this variation can only be detected in situations (such as those involving cerebral trauma or cerebrovascular accidents) that are incompatible with continued existence in a natural setting. It is to be hoped that further work will clarify the question.

SUMMARY

The arteries of the base of the mouse brain were examined after perfusion with India ink. A qualitative difference exists between inbred mice of three strains (C57BL/6J,

129/J and BALB/cCF) on the one hand, and genetically defined heterogeneous mice on the other; the latter consistently show anomalies similar to those previously described in genetically undefined rodents, whereas inbred mice do not. A quantitative morphometric analysis of the Circle of Willis of inbred mice was undertaken. The results of this analysis are consistent with the notion that the differences in shape between the circles of Willis of different strains of inbred mice are due to additive genetic variation between these strains.

This work was supported by the Natural Sciences and Engineering Research Council of Canada, the Fonds Institutionnel de Recherche de l'Université du Québec, and Grant GM 23618 from the National Institutes of Health. Its contents are solely the responsibility of the Université du Québec à Trois-Rivières and the Jackson Laboratory, and do not necessarily represent the official views of NIH. The Jackson Laboratory is fully accredited by the American Council on Laboratory Animal Care.

REFERENCES

ADACHI, B. (1928). *Das Arteriensystem der Japaner*. 2 vols. Kyoto: Verlag der Kaiserlich-Japanischen Universität zu Kyoto.

ALPERS, B. J., BERRY, R. G. & PADDISON, R. M. (1959). Anatomical studies of the Circle of Willis in normal brain. *Archives of Neurology and Psychiatry* **81**, 409–418.

BAILEY, D. W. (1985). Genes that affect the shape of the murine mandible: congenic strain analysis. *Journal of Heredity* **76**, 107–114.

BAILEY, D. W. (1986). Genes that affect morphogenesis of the murine mandible: recombinant-inbred strain analysis. *Journal of Heredity* **77**, 17–25.

BROWN, J. O. (1968*a*). The morphology of Circulus arteriosus cerebri in rats. *Anatomical Record* **156**, 99–106.

BROWN, J. O. (1968*b*). Some observations on the cerebral arterial circles of mink (*Mustela vison*). *Anatomical Record* **161**, 311–324.

BUGGE, J. (1974). The cephalic arterial system in insectivores, primates, rodents and lagomorphs, with special reference to the systematic classification. *Acta anatomica* **87**, *Suppl* 62.

COLLINS, R. L. (1985). On the inheritance of the direction and degree of asymmetry. In *Cerebral Lateralization in Nonhuman Species* (ed. S. D. Glick), pp. 41–70. New York: Academic Press.

EDMONDS, H. W. & SAWIN, P. B. (1936). Variation of the branches of the aortic arch in rabbits. *American Naturalist* **70**, 48–49.

FALCONER, D. S. (1981). *Introduction to Quantitative Genetics*, 2nd ed. London: Longmans.

FESTING, M. F. W. (1976). Phenotypic variability of inbred and outbred mice. *Nature* **263**, 230–232.

FESTING, M. F. W. & LOVELL, D. P. (1981). Domestication and development of the mouse as a laboratory animal. In *Biology of the House Mouse* (ed. R. J. Berry), pp. 43–62. London: Academic Press.

FIRBAS, W., SINZINGER, H. & SCHLEMMER, M. (1973). Ueber den Circulus arteriosus bei Ratte, Maus und Goldhamster. *Zentralblatt für Veterinärmedizin, Reihe C* **2**, 243–251.

FROUD, M. D. (1959). Studies on the arterial system of three inbred strains of mice. *Journal of Morphology* **104**, 441–478.

GREENE, E. C. (1935). Anatomy of the rat. *Transactions of the American Philosophical Society*, **27**. Reprinted in 1963 by Hafner, New York.

HASEBE, K. (1928). Arterien der Hirnbasis. In *Das Arteriensystem der Japaner* (ed. B. Adachi), Band I, pp. 111–134. Kyoto: Verlag der Kaiserlich-Japanischen Universität zu Kyoto.

KAHAN, B., AUERBACH, R. ALTER, B. J. & BACH, F. H. (1982). Histocompatibility and isoenzyme differences in commercially supplied "BALB/c" mice. *Science* **217**, 379–381.

MCNUTT, C. W. & SAWIN, P. B. (1943). Hereditary variation in the vena cava inferior of the rabbit. *American Journal of Anatomy* **72**, 259–288.

MORSE, H. C. (1978). *Origins of Inbred Mice*. New York: Academic Press.

RODERICK, T. H. (1980). Strain distributions of genetic polymorphisms in the mouse. In *Handbook on Genetically Standardized JAX Mice*, (ed. H. J. Heiniger & J. Dorey), pp. 2.22–2.29. Bar Harbor, Maine: The Jackson Laboratory.

SAKUMA, F. (1961). Studies on the cerebral arteries of *Macacus cyclopsis*. *Acta medica nagasakiensia* **5**, 160–181.

SAWIN, P. B. & EDMONDS, H. W. (1949). Morphogenetic studies of the rabbit. VII. Aortic arch variations in relation to regionally specific growth differences. *Anatomical Record* **105**, 377–397.

SAWIN, P. B. & NACE, M. A. G. (1948). Morphogenetic studies of the rabbit. V. Inheritance of an asymmetrical vascular pattern. *Journal of Morphology* **82**, 331–354.

SOKAL, R. R. & ROHLF, F. J. (1981). *Biometry*, 2nd ed. San Francisco: W. H. Freeman.

STAATS, J. (1966). The laboratory mouse. In *Biology of the Laboratory Mouse* (ed. E. L. Green), pp. 1–9. New York: McGraw-Hill-Blakiston.

TAYLOR, B. A. (1972). Genetic relations between inbred strains of mice. *Journal of Heredity* **63**, 83–86.

WARD, R. (1985). Genetic polymorphisms and additive genetic models. *Behavior Genetics* **15**, 537–548.