AN ASSOCIATION OF HEREDITARY EYE DEFECTS WITH WHITE SPOTTING

BY L. C. DUNN AND JAN MOHR*

COLUMBIA UNIVERSITY

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In a paper in these PROCEEDINGS[1] an asymmetrical effect on eye color in the house mouse was ascribed to a new mutant gene, ruby eye (*ru*). It was noted, however, that almost all mice in which the two eyes differed in color, referred to as *heterochromia iridis*, showed piebald spotting. This connection between eye color variation and spotting has now been studied further with the result that the eye color modification has been shown to be due to specific defects of the iris, which in turn appear to be effects of a mutant gene, *s*, for piebald, which has long been known in laboratory mice. A strain of ruby-eyed mice without spotting has been observed for over twenty generations and no case of heterochromia has been seen.

Evidence for the association of iris defects and spotting will be given below, together with a preliminary description of the iris abnormalities derived from gross examinations with an ophthalmoscope. All animals in these experiments were thus observed at the time of weaning (3–4 weeks of age) and the diagnosis was in many cases confirmed by later examinations. A careful microscopic study of the defects remains to be made.

Anisocoria in Piebald Mice.—Many of the mice in our inbred strain An (= aniridia: black-and-tan silver ruby piebald $a^t a^t$ *BB ruru sisi ss*) show noticeable differences from the normal ruby eye color. One or both eyes may be light ruby or pale pink. When these variant eyes are examined with an ophthalmoscope the commonest finding is an enlarged pupil (anisocoria) varying all the way from slight enlargement to what appears to be complete absence of the iris (aniridia). Enlargement of the pupil is generally accompanied by reduction of the amount of pigment in the iris with consequent approach to the pink color of unpigmented eyes. A smaller proportion of lighter colored eyes have pupils of normal or nearly normal size but lack a part or all of the dark iris pigment. Occasionally a segment of iris is unpigmented. Out of 266 eyes of this strain examined in mice of the sixth and seventh inbred (brother-sister) generations, 120 had the iris reduced (98) or missing (22); 48 without gross iris defects had pigment reduced (34) or missing (14); while the remaining 98 had no gross defects. Of the 133 animals examined, both irides were normal in only 28, so that in this strain the iris defect expresses itself in about 80 per cent of the individuals and in some 63 per cent of the eyes. Thus there is a high degree of asymmetry in gross expression. The right and left eyes have about the same probability of being abnormal as judged by ophthalmoscopic ex-

amination. The proportion of eyes with histological abnormalities is unknown. The differences in iris character between two sides of the same individual and between individuals of this inbred strain are apparently non-genetic.

In contrast with this strain is another of similar derivation. This strain (Anophthalmic), now in its 22nd brother-sister generation, has a genotype which is similar to that of the Aniridia strain except that it lacks the piebald gene (*a^{t}a^t BB ruru sisi*). It contains a gene or genes which express themselves in some 65 per cent of the individuals, often asymmetrically, by causing a defect of the bulbus and/or cornea which varies from bilateral anophthalmia to unilateral staphyloma. In none of the eyes of this strain with normal corneae have the iris defects of the Aniridia strain been seen.

Tests of Inheritance of Anisocoria.—The most direct test of the hypothesis that anisocoria is an effect of the piebald gene is to cross the above two strains which differ primarily at the piebald locus. From this cross 26 F_1 animals were examined. All were non-piebald with ruby eyes and normal irides.

Fourteen of these F_1's were backcrossed to the spotted ruby (An) strain and produced the results shown below:

	NORMAL IRIDES	ABNORMAL IRIDES	TOTAL
Non-piebald	116	4	120
Piebald	72	43	115

Of the non-piebald offspring only 3.3 per cent had any abnormality of the iris, and of these one had an oval pupil on one side, and one had a slightly enlarged pupil on one side. Of the other two, one had no detectable iris on one side; the other had an unquestionably enlarged pupil on one side. These last two cases are exceptions to the rule that anisocoria appears only in piebald animals. They may of course have been homozygous for the piebald gene *ss* which for some reason did not express itself in spotting, but there is no experimental test of this possibility. Two cases of what then was called heterochromia iridis were noted among 48 non-piebald ruby eyed mice.[1] Although these were not examined with the ophthalmoscope, they were probably exceptions of the sort noted above.

The significant feature of the results was the appearance of iris abnormalities in some 37 per cent of the piebald animals. This is not far from the frequency of iris abnormalities in the An piebald strain at the time (F_3) when the cross was made (15 out of 32 animals examined showed gross abnormalities of the iris).

The data show that piebald spotting and iris defects are closely associated and do not exclude the hypothesis that they are due to the same genotypic cause.

Anisocoria in Other Spotted Strains.—If the iris defects are due to the

action of spotting genes they should be found in other spotted stocks unrelated to the above. Among 39 piebald mice from an inbred black Brachy line in this laboratory known to be genetically *ss*, 5 cases of small or absent iris were found. Among 63 piebald animals of Line 3 (Black agouti) in this laboratory, eleven had gross defects of the iris. One case was found among 95 animals with spotted face in Dr. Schneider's colony at Rockefeller Institute for Medical Research and one case among 15 animals with the "splotch" mutant condition in strain C57 black. The last two strains are not known to carry the piebald gene. These 18 cases were all found in black-eyed animals so that although the ruby-eye phenotype makes the iris abnormality easier to identify with the naked eye, ruby is not a necessary condition for its development. In all of the 102 black-eyed piebald animals from the Brachy line and Line 3 a deficiency of the chorioidal pigmentation could be recognized ophthalmoscopically. This confirms earlier findings of defective chorioidal pigmentation in piebald mice.[2,3] In ruby animals this deficiency is difficult to recognize ophthalmoscopically, but histological examination indicates that it is a general feature of the piebald phenotype in these animals too.

Test crosses between a piebald with abnormal iris from Line 3 and one from strain An produced 5 spotted animals with abnormal iris and 9 spotted without iris abnormality, showing a similar genetical basis for anisocoria in those two unrelated strains. A test of one anisocoric animal from the Brachy strain by strain An was negative yielding only 15 animals with normal irides. Because of the small numbers involved, this test was not conclusive for the question at issue.

Among the 176 self-colored animals from a variety of strains, examined with the ophthalmoscope, not a single case of anisocoria was found. However, 15 cases of abnormal iris were found among 100 albinos examined from various strains. Six of these cases, all from an inbred albino Fused strain of this laboratory, had coloboma iridis and were thus quite different from the anisocoria associated with piebald. The Fused strain was tested and found not to carry piebald. These and other cases of eye abnormalities found in several strains will provide good material for ophthalmological study.

Finally, a new occurrence of Aniridia in several related animals from a mixed stock has been observed. In the first case, a brown piebald animal, both eyes were light pink. When tested by *ruru SS* this animal was shown to have the dominant allele of ruby (Ru), giving 26 non-ruby in F_1, and matings among F_1 animals according to birth classification showed segregation, for uncolored irides (80 colored, 29 uncolored) for one eye color mutant only, viz., ruby eye.

In F_2 and backcross progenies, ophthalmoscopic examination of irides at weaning revealed several cases of anisocoria including aniridia, but these

occurred only among spotted animals. Since the case was apparently similar to that analyzed above it was not studied further. It does show, however, that although anisocoria is easier to detect due to the pinkness of the eye, when it occurs in ruby-eyed animals, it may exert its full effect in spotted animals without the ruby mutation and thus simulate a mutation.

Discussion.—It is evident from the above that the type of related iris abnormalities first noted in strain An and shown there to be associated with the piebald condition are found also in other unrelated piebald strains of various genotypes and rarely in spotted strains not known to carry the gene *s*.

This is probably not the first time that eye defects have been noted in piebald mice, although it is the first time that their genetic nature has been analyzed. Durham[2] in 1908 noted "ruby eyes" in three mice. In each case the choroid pigment was greatly reduced, and in each case the abnormal individual was chocolate (brown) *piebald*. No information on size of pupil was given. Later Gates[3] examined a population segregating for piebald, agouti, black-brown, and density and dilution, and found that all spotted animals and only spotted animals lacked chorioidal pigmentation. External defects, such as pinkness or heterochromia, were not noted. The present study indicates also that in piebald mice the chorioidal pigmentation is generally defective.

There is thus a general association between spotting and eye pigmentation which suggests the following working hypothesis: The genotype responsible for piebald spotting (and perhaps for certain other spotting types) produces its effect by increasing the probability of death or failure in function of the pigment cells (melanophores). In the mouse these cells are derived from the neural crest (Rawles[4]) and reach their destinations by migration. The normal development of the iris depends upon actual or inductive contributions by such cells. If their probability of survival is lowered, the failure of pigment development in local areas of the skin will be accompanied by failure to invade the choroid, and an increased probability of failure of development of the stroma of the iris or of its pigment or of both. In some such manner as this the effect of a "spotting gene" upon a structural component such as the iris may be envisaged.

Since both amiridia and spotting occur as genetic variations in man, a study of their relations in human families may yield some evidence on the hypothesis suggested.

* Visiting Fellow of the Rockefeller Foundation, 1949; now at Genetics Institute, The University, Oslo, Norway.

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