STUDIES ON AN ANOPHTHALMIC STRAIN OF MICE. IV. A SECOND MAJOR GENE FOR ANOPHTHALMIA

HERMAN B. CHASE

Department of Zoology, University of Illinois, Urbana, Illinois

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INTRODUCTION

INHERITANCE of eyelessness in mice has been discussed in a previous paper (CHASE 1942b) in which the method of analysis was the crossing of the inbred eyeless strain with other strains. The present study considers another method suggested at that time, involving a control of modifiers. This was made possible by a reverse mutation toward normal eyes in the eyeless strain. From the mutant individual two sublines have been developed, one with 98 percent anophthalmic mice, the other with 96 percent normals (preliminary report CHASE 1942a).

The fundamental phenomenon in the embryology of the eyeless strain is the inhibition of the growth of the optic vesicle at ten days or earlier, just after the normal formation of the vesicle (CHASE and CHASE 1941). Because of variations in the degree of inhibition, ten percent of the original strain have "small" or "medium" eyes as observed at birth or postmortem.

From the previous study (CHASE 1942b) of crosses of anophthalmic strain B with strain C57 Black and with strain K, one major factor difference, *ey* recessive for eyelessness, was deduced. From results of crosses with strains L and H, two hypotheses were suggested. In one hypothesis two major factors were present with one dominant sufficient for normal eyes (double recessive necessary for eyelessness) and strong minus modifiers from strain B. In the other hypothesis there was one major factor with dominance of normal eyes and strong plus modifiers from L and H. The present paper will give additional evidence that the original anophthalmic strain is homozygous for one major factor, *ey ey*, and will present data to show that it is homozygous for another major factor for anophthalmia, present also in C57 Black but not in L and H.

HISTORY OF MUTANT

The anophthalmic strain B has the genotype *CCAAbbddSS*. The origina animals (from strain R) were obtained in 1938 from DR. C. C. LITTLE of the ROSCOE B. JACKSON MEMORIAL LABORATORY at Bar Harbor, Maine. LITTLE observed reduced eyes in offspring from a wide cross, and selected for this character in subsequent generations. Twenty-one successive generations (16 at Illinois) of brother-sister matings have now been made. Birth records with confirmatory postmortem examinations have been employed for any study involving this character. In this strain, 88.3 percent are completely eyeless, 2.7 percent have very small eyes (both eyes or very small on one side and eyeless on the other), and 9.0 percent have intermediate eyes (for instance small left and eyeless right or small on both sides, etc.). Only a very small number of those with intermediate eyes have eyes large enough to be seen externally in the

adult. No normal eyes occur. The relatively few animals having one or both eyes of the very small category are combined with the completely eyeless animals as they were in the previous paper on the inheritance of eyelessness. Justification for this procedure is found in the embryological observations and in the breeding results from crosses with other strains where very small and eyeless behave similarly and not like small and medium.

In the eleventh generation of successive brother-sister matings (sixth at Illinois) a female appeared with a normal left eye and medium right eye, a condition much nearer normality than was every recorded in this strain. No fault in the record is possible because this female had the typical dilute brown agouti color characteristic of the strain, and crosses with any other animals present in the colony at that time would have given a different color. This female, mated with a completely eyeless brother, produced offspring indicating that she was heterozygous for a factor for normal eyes. From this mating two sublines have been selected, one with 98 percent anophthalmics and the other with 96 percent normals.

The two sublines derived from the mutant female (normal left and medium right) crossed with her eyeless brother are named Be_1 for the 98 percent eyeless group and Be_2 for the 96 percent normal group. By successive brother-sister matings Be_1 has reached the seventh generation and Be_2 has reached the fifteenth generation beyond the original mutant. After the third generation these two selected sublines have remained constant in expression. The rapidity of fixation indicates that at most very few inherited modifiers were heterozygous at the time selection for the two sublines started.

Line B was continued through the eyeless sister of the mutant and the same eyeless male.

CROSSES WITH OTHER STRAINS

The development of the two sublines is presented in table 1, and also the results of crosses of Be_2 (normal subline) with other strains are indicated. Since no significant differences were found between males and females, the sexes are combined in this paper.

In the crosses involving C57 Black, the subsequent F_2, and backcrosses to Be_2, it appears that Be_2 and C57 Black do not possess the same alleles for normal eyes and that modifiers alter the ratios. More information is gathered from the two crosses presented at the bottom of table 1. The original mutant female crossed with a normal male which was an F_1 individual from strain B by C57 Black (therefore *Ey ey* in genotype) produced 63.7 percent normal offspring. A similar reciprocal cross of an F_1(B$\times$C57 Blk) female by a son of the original mutant produced 62.8 percent normal offspring. This evidence suggests either a new intermediate allele or, more probably, a new independent factor for normal eyes not showing dominance.

All offspring from the series of crosses involving Be_2 with strains L and H are normal. When this result is compared with the segregating results of similar crosses involving B with L and H (CHASE 1942b), it strengthens the suggestion of a new independent factor for normal eyes rather than a new

TABLE 1

Percentage of animals with normal eyes, intermediate eyes, and no eyes. L=left eye, R=right eye, norm=normal, med=medium, eyl=eyeless, inter=intermediate.

	PERCENTAGE			TOTAL NUMBER
	NORMAL	INTER-MEDIATE	EYELESS	
Strain B		9.0	91.0	1054
Strain Be				
Mutant norm L med R ♀×eyl ♂	7.7	38.5	53.8	39
F_2, norm L med R ♀×norm ♂	17.8	35.6	46.6	45
F_3, norm L med R ♀×norm ♂	83.3	16.7		30
F_3, norm ♀×norm ♂	87.0	8.7	4.3	23
F_2, eyl ♀×small L and R ♂		43.2	56.8	102
F_3, eyl ♀×eyl ♂		10.0	90.0	50
Be_1, F_4–F_8, eyl ♀×eyl ♂		2.3	97.7	343
Be_2, F_4–F_{16}, norm ♀×norm ♂	96.6	3.3		447
Be_2, F_6, inter ♀×norm ♂	100.0			28
Strain C57 Black	95.7	4.3	0.1	2200
Be_2×C57 Black	100.0			43
F_2	89.8	7.8	2.3	128
Backcross to Be_2	85.2	11.6	3.1	129
Second backcross to Be_2	78.3	21.7		23
Strain L	100.0			>1500
Be_2×L	100.0			22
F_2	100.0			99
Backcross to Be_2	100.0			19
Strain H	100.0			>1500
Be_2×H	100.0			32
F_2	100.0			85
Backcross to Be_2	100.0			42
Second backcross to Be_2	100.0			4
Mutant ♀×F_1 (B×C57 Blk) norm ♂	63.7	27.3	9.0	22
F_1 (B×C57 Blk) ♀×norm F_2 Be ♂	62.8	37.2		43

allele of *ey*. This normal subline derived from the mutant therefore makes it possible to decide between the two hypotheses advanced in the previous paper to explain the difference in results of crosses between anophthalmic strain B and normal strains L and H compared with crosses with C57 Black. Two major genes for normal eyes are present in L and H and only one of them in C57 Black.

CROSSES BETWEEN ANOPHTHALMIC SUBLINE AND NORMAL SUBLINE

Crosses between the Be_1 anophthalmic subline and the original B anophthalmic strain give results which agree reasonably with the regular B frequency of approximately 90 percent eyeless (table 1). Because of this, any great differ-

ence of modifiers between these strains may be eliminated from consideration. As the two inbred sublines, Be_1 (98 percent anophthalmic) and Be_2 (96 percent normal), are derived from the original mutant female of the eleventh generation of successive brother-sister matings, they offer a method of analysis of inheritance wherein the modifiers must be nearly the same and the major factor to be studied represents the only difference. Eyeless by eyeless matings in the F_2 and F_3 of these subline crosses do not all give approximately 98 percent eyeless (table 2). This fact must be due to the fact that eyeless phenotypes do not always correspond with genotypes homozygous for both alleles for anophthalmia.

TABLE 2

Crosses between the two sublines, Be_1 anophthalmic and Be_2 normal. Eyl = eyeless, inter = intermediate, norm = normal, BX = backcross, ditto = same animal.

CROSS	♀	♂	PERCENTAGE			TOTAL NUMBER
			NORMAL	INTER-MEDIATE	EYELESS	
F_1	eyl	norm	14.8	72.2	13.0	54
BX	eyl	"	78.6	21.4		14
F_2	norm	eyl	17.4	60.9	21.7	23
F_2	eyl	"	9.1	81.8	9.1	11
F_3	norm	eyl	9.8	46.3	43.9	41
F_3	eyl	"		34.6	65.4	26
F_1	norm	eyl	52.6	47.4		38
BX	norm	"	5.0	40.0	55.0	20
BX	inter	"		29.4	70.6	17
F_2	inter	inter	30.0	47.5	22.5	40
F_2	inter	"	28.6	39.3	32.1	56
F_3	norm	eyl	15.3	83.0	1.7	59
F_3	eyl	"	2.1	33.3	64.6	48

No dominance is found for normal or eyeless when these two sublines are crossed. Furthermore, the heterozygote (table 2) has a complete range of expression from normal to eyeless and the two homozygotes both overlap into the intermediate group and possibly rarely with each other.

One non-hereditary modifier, a matroclinous tendency, was suspected early in this study. Normal mothers tend to shift the median of the range of expression toward normality in their offspring and eyeless mothers, toward eyelessness. Analyzed by "Student's" paired comparison method, the probability is .08, a border-line situation and suggestive only of a matroclinous tendency.

DISCUSSION

In CHASE (1942b) two hypotheses were suggested for the difference between the ratios of B by C57 Black crosses and of the B by L or H crosses. One hypothesis involved one major factor difference for both types of crosses and

greatly different modifiers to explain the ratio differences. The other hypothesis involved two major factors to explain the results of L and H crosses, as compared with C57 Black. By the latter hypothesis only one allele for normal eyes would be sufficient to produce normality. This is an example of duplicate factors (15:1).

Two inbred sublines, one anophthalmic and the other normal, were derived from the mutant female which occurred in the eleventh generation of successive brother-sister matings. Modifiers are thus controlled and an analysis of this new gene can be made. It shows no dominance, and the variability of expression is such that the heterozygote ranges from normal to eyeless, and both homozygotes overlap into the intermediate category. This lack of dominance of the second major factor and dominance of the first major factor agree extremely well with the B by L and H crosses (CHASE 1942b). For instance there were 88.7 percent normals in the F_2 generations (522 animals) rather than the 93.75 percent (15:1) to be expected with complete dominance of both major factors. Furthermore, C57 Black, having the normal allele of the first major gene for defective eyes, crossed with Be_2, having the normal allele of the second major gene, gives in the F_2 generation a percentage of 89.8 normals. This close agreement with the B by L and H F_2 is clear evidence that L and H have both major alleles for normality and B has the corresponding major alleles for anophthalmia.

The new gene appears from tests not to be an allele of the previously reported gene for anophthalmia but rather an independent factor. The original gene for anophthalmia *ey* must be named *ey-1* and the new mutant *Ey-2*. From crosses between various lines, the genotypes in relation to these genes for anophthalmia are:

Strain B, original anophthalmic	*ey-1 ey-1; ey-2 ey-2*
Subline Be_1, anophthalmic	*ey-1 ey-1; ey-2 ey-2*
Subline Be_2, normal	*ey-1 ey-1; Ey-2 Ey-2*
Strain C57 Black, normal	*Ey-1 Ey-1; ey-2 ey-2*
Strains L and H, normal	*Ey-1 Ey-1; Ey-2 Ey-2*

Of general interest for the field of genetics is the observation that eyelessness depends on the cumulative effect of two mutant alleles. When animals of the eyeless strain are crossed with normal strains L and H, there is in the F_2 a 15:1 ratio. This ratio, characteristic of duplicate factors, is very unusual among animals. Also of general interest is the occurrence of a reverse mutation in a mammal and the fact that it does not show dominance in the combinations tested, whereas *Ey-1* does show dominance.

SUMMARY

Ey-1 shows dominance over *ey-1*, the gene originally described for anophthalmia.

A mutant female occurred in the original anophthalmic strain. This female had a normal left and a medium right eye, far beyond the usual range of the anophthalmic strain.

Two sublines were developed from the mutant, an anophthalmic subline and a normal subline.

Ey-2, the new mutant gene, shows no dominance over *ey-2*, and the heterozygote ranges from normal to eyeless.

Normal eyes may occur in the presence of either *Ey-1* or *Ey-2*.

The allele *Ey-2* is homozygous in strains L and H but absent (*ey-2 ey-2*) in C57 Black, whereas *Ey-1* is homozygous in all three strains.

Both separate alleles for anophthalmia are present in strain B and in the anophthalmic subline (*ey-1 ey-1; ey-2 ey-2*).

The *Ey-2* allele for normal eyes and the *ey-1* for eyelessness are present in the normal subline.

This case seems to be one of the few examples of duplicate factors (15:1 ratio) found in animals.

The mutant allele *Ey-2* is a reverse mutation, an occurrence probably rare in mammals.

LITERATURE CITED

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