INHERITANCE OF PIGMENTED LARVAL HEAD CAPSULES IN *CULEX PIPIENS* [1]

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In a search for naturally occurring mutants in *Culex pipiens,* a factor causing increased pigmentation of the larval head capsule was discovered (VandeHey, 1964). The present paper gives a detailed description and analysis of the mode of inheritance for this mutation, designated *Black larva, Bl.*

The *Bl* mutant was discovered in the fourth generation of an inbred line derived from a female collected by A. Wild in a home near Mainz, Germany. All of the larvae from a single-pair mating possessed distinctively dark head capsules (Fig. 1). Normally, in *C. pipiens,* the exoskeleton of the larval head capsule is translucent, or has only a small pigmented bar on each gena (Fig. 2). This pigmented bar was greatly enlarged in the *Bl* mutants. In addition, the frons and labial areas were heavily pigmented. The dark color made it easy to distinguish the mutant forms from normal individuals. Although all larval instars were pigmented, separation of the phenotypes was most easily accomplished with third and fourth instars.

After two generations of mass culture inbreeding, mutant forms were single-pair mated with individuals from another normal inbred line in order to determine the mode of inheritance. Among progeny of these crosses it was observed that heterozygous females could be detected if the larval exuviae were examined microscopically (Fig. 3). There was some increase of pigmentation on the labium. Since the ventral surface of the head capsule is rarely exposed to view in swimming larvae, it was difficult to distinguish these heterozygous females from normal females on gross examination. Males showed either full expression or none at all.

Figure 4 and table 1 present the schema of crosses and the results obtained. According to Gilchrist and Haldane (1947), who showed that sex is determined as a single allele in *Culex,* the symbols m/m and m/M are used for female and male respectively. The inheritance of *Bl* appears to be that of a sex-linked dominant gene with sex-influenced expression. When *Bl* males were outcrossed, all of the progeny appeared light in color (table 1, line 1). Microscopic examination of some larval exuviae revealed that the females were heterozygous. However, since such microscopic examination of each larva was too time-consuming, these heterozygous females were classed with the light or normal females. The male progeny appeared light on gross and microscopic examination. In the reciprocal cross, when *Bl* females were outcrossed, the male progeny had dark head capsules while the female progeny had light head capsules (line 2).

Inbreeding of the progeny from the above outcrosses produced two different results. In cases where the progeny were derived from an outcross of a mutant male, approximately one-half of the F_2 males were phenotypically *Bl* while all of the F_2 females appeared normal (line 3). On theoretical grounds it is assumed that one-half of these F_2 females must have been heterozygous for the trait. In cases where the progeny were derived from an outcross of a mutant female, approximately one-half of both F_2 males and females were phenotypical mutants (line 4).

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Regardless of their source, backcrosses of the F_1 females to the mutant stock

Fig. 1–3.—Exuviae of fourth instar larval head capsules. Fig. 1—mutant phenotype (homozygous female); Fig. 2—normal phenotype; Fig. 3—phenotype of heterozygous female.

gave similar results: one-half of both males and females were easily identified as mutant phenotypes (lines 5 and 6). However, backcrosses of the F_1 males gave two different results. If the F_1 males were derived from an outcross of a mutant male, the female backcross progeny were light (heterozygous) and the males were dark (line 7). If the F_1 males were derived from an outcross of a mutant female, all of the backcross progeny, males and females, were dark (line 8).

F_1 individuals derived from the reciprocal outcrosses (lines 1 and 2) were also backcrossed to the inbred normal line. When the F_1 males were backcrossed in this manner, all of the progeny appeared normal (lines 9 and 10). On the other hand, when the F_1 females were backcrossed to the normal line, the female progeny appeared normal on macroscopic examination but one-half of the male progeny appeared dark (lines 11 and 12).

Chi-square tests fall within the expected values with the exception of line 3, for which P is less than 0.01, and line 6, for which P lies between 0.05 and 0.01. In both of these cases there was a slight predominance of males over females and normal over mutant phenotypes. There seemed to have been a general tendency in this direction for most of the crosses. Tests for heterogeneity (Bailey, 1961) show no significant variations in the results of any given genotypic class of crosses.

In several cases, a few unexpected phenotypes were recovered. These are the individuals recorded in parentheses in table 1. They may have been the result of errors in classification or of phenotypic variations caused by modifying factors. Several of the "light" males of line 2 were crossed with light females obtained from the same line. The ratio of light to dark progeny was nearly 1:1 among both males and females, (40:45, males; 54:52, females). Somewhat similar results were obtained when the two "dark" females of line 2 were crossed with dark males from

the same line (44:47, males; 45:28, females). This indicated that these exceptional individuals were similar in genotype to the other progeny recorded in line 2. There was no time available to run further tests on these or on exceptional individuals from the other lines.

The Bl mutation also had a pleiotropic effect on the rate of development. In general, pupation and adult emergence was delayed approximately 4 to 5 days. Figure 5 shows the composite daily record of adult emergence obtained from one type of backcross $(Bl,m/+,m \times Bl,m/+,M)$. The days are numbered from the day on which the first adults emerged in the single-pair cultures. It is evident that mutant males took an average of 5 days

TABLE 1.—Results of crosses determining the mode of inheritance for a factor causing black head capsules in larvae of *Culex pipiens*.

Type of cross	Number of pair matings	Male Light	Male Dark	Female Light *	Female Dark	X^2
1. $\frac{+m}{+m} \times \frac{Bl\,m}{+M}$	10	238	...	206	...	2.3
2. $\frac{Bl\,m}{Bl\,m} \times \frac{+m}{+M}$	11	(6)**	276	283	(2)	0.2
3. $\frac{+m}{Bl\,m} \times \frac{+m}{+M}$	12	228	171	351	(1)	11.7†
4. $\frac{Bl\,m}{+m} \times \frac{Bl\,m}{+M}$	13	186	163	199	155	7.1
5. $\frac{+m}{Bl\,m} \times \frac{Bl\,m}{+M}$	9	131	144	132	119	2.4
6. $\frac{Bl\,m}{+m} \times \frac{Bl\,m}{+M}$	14	273	232	260	214	8.7‡
7. $\frac{Bl\,m}{Bl\,m} \times \frac{+m}{+M}$	9	(2)	224	231	(1)	0.1
8. $\frac{Bl\,m}{Bl\,m} \times \frac{Bl\,m}{+M}$	13	...	243	...	210	2.4
9. $\frac{+m}{+m} \times \frac{+m}{+M}$	9	183	(2)	145	...	4.4
10. $\frac{+m}{+m} \times \frac{Bl\,m}{+M}$	6	115	...	89	...	3.3
11. $\frac{+m}{Bl\,m} \times \frac{+m}{+M}$	6	109	105	224	(1)	0.3
12. $\frac{Bl\,m}{+m} \times \frac{+m}{+M}$	11	165	178	329	(7)	0.9

* The light females include both the homozygotes ($+,m/+,m$) and the heterozygotes ($+,m/Bl,m$ or $Bl,m/+,m$). See text for explanation.

** Numbers in parentheses include exceptional individuals whose phenotype may be due to modifying factors.
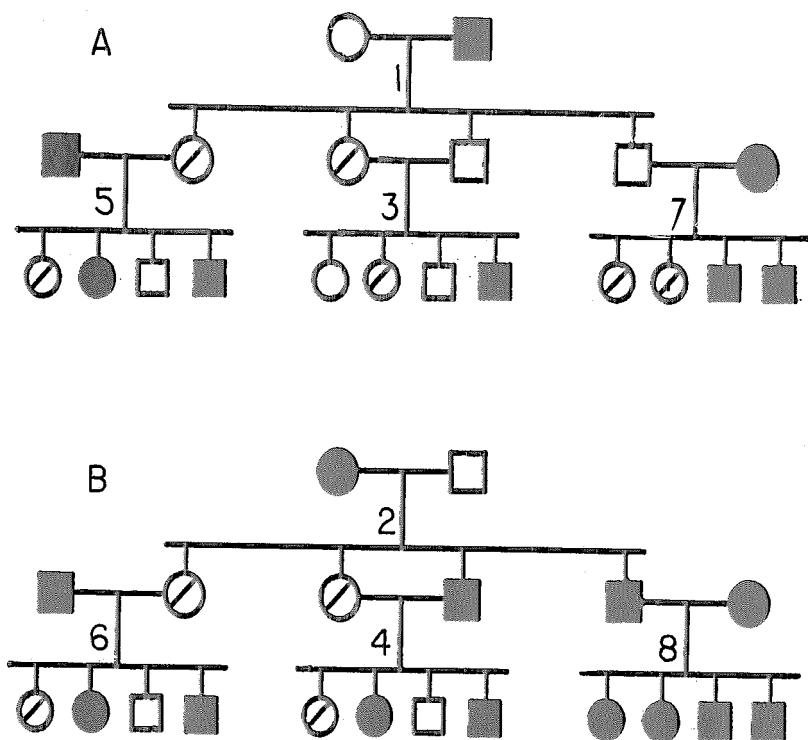
† P<0.01.

‡ 0.05>P>0.01.

Fig. 4—Schema of crosses made to determine mode of inheritance for Black larva (*Bl*) in *Culex pipiens*. Schema A—outcross of *Bl* male with F₂ and reciprocal backcrosses. Schema B—outcross of *Bl* female with F₂ and reciprocal backcrosses. Circles denote females; squares, males; open figures, light phenotypes; solid figures, mutant phenotypes; circles with diagonal line, heterozygous females which could be detected microscopically. Numbers correspond with the lines in table 1.

longer than normal males to reach adult emergence and the homozygous females took an average of 4 days longer than the heterozygous females.

The effect on developmental rate is especially noteworthy when comparing the results of the reciprocal outcrosses of the mutant stock (fig. 6). Normally males pupate and emerge before the females in *C. pipiens*. While this was true of the progeny obtained from the outcross of the mutant males $(+,m/+,m \times Bl,m/+,M)$, among the progeny of the reciprocal cross $(Bl,m/Bl,m \times +,m/+,M)$ most of the females emerged before the males. It should be noted that males from the former cross were genotypically normal,

males from the latter cross were heterozygous mutants, and females from both crosses were heterozygous.

In summary, *black larva* (*Bl*) is inherited as a sex-linked factor showing complete dominance in the male and incomplete dominance in the female. Penetrance is nearly complete and there is little variation in expressivity. Finally, the mutation shows the pleiotropic effect of decreasing developmental rate.

On a practical note, the mutation could be a valuable tool for genetic and physiological experiments. Crossing a stock to be studied with homozygous *Bl* females would permit early separation of males from females on the basis of color of head
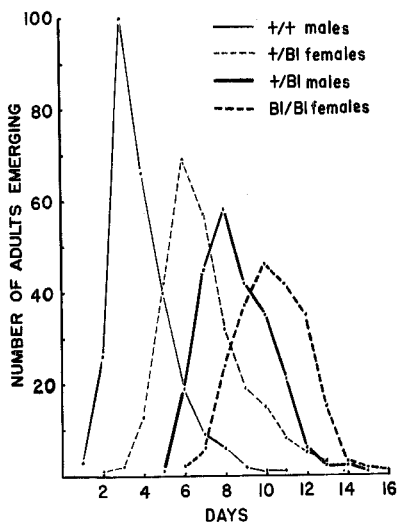
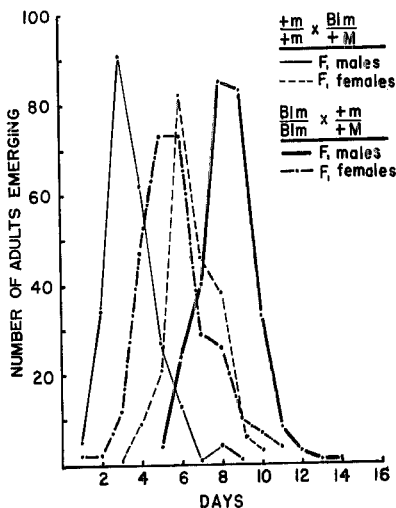FIG. 5.—Number of adults emerging per day from the backcross, $Bl,m/+,m \times Bl,m/+,M.$

FIG. 6.—Number of adults emerging per day from outcrosses of Bl males $(+,m/+,m \times Bl,m/+,M)$ and females $(Bl,m/Bl,m \times +,m/+,M).$

capsules in the third or even second or first larval instar. It should also be interesting to study the linkage relationship of Bl with other sex-linked genes (*white, red, var*) as described by Laven (1957), Gilchrist and Haldane (1947) and Wild (1963).

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