I. A STUDY OF A MUTATION
IN
DROSOPHILA MELANOGASTER.

II. A SYSTEMATIC STUDY OF THE LEECHES
OF WESTERN CANADA.

by

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A STUDY OF A MUTATION IN DROSOPHILA MELANOGASTER.

HISTORICAL:

Drosophila, melanogaster, commonly known as the fruit or vinegar fly, has been used in various biological experiments since 1905 when Carpenter studied its reactions to light, to gravity, and to agitation. In 1906 Castle, Carpenter, Clark, Mast and Burrows published the results of their investigations on the effect of inbreeding. Their work showed that there is no decrease in the fertility of an individual as a result of close inbreeding. In 1907 Lutz reported the result of some observations on the inheritance of a wing-vein modification. In 1910-11 a paper by Delcourt and Guyénet appeared which dealt with the effect of food conditions on Drosophila. The question of the effect of inbreeding on fertility was also studied by Moenkhaus. He also made observations on the variations of the sex-ratio in relation to selection. This work was published in 1911.

The work then, up to this time, had practically all been of a non-genetical nature for the value of D. melanogaster for genetic work had not as yet been realized. However, with the appearance of the first mutant the work took a new turn, for this species has proved to be of inestimable value in the field of genetics. Firstly, it thrives under laboratory conditions; secondly, its short life cycle makes it possible to raise many generations in a comparatively short period of time; thirdly, the chromosome number is very small, and, lastly, the form has given rise to many mutations. Through his work with this fly, Dr. Morgan has brought the chromosome theory of heredity to its present stage.

TECHNIQUE:

The flies were raised on banana-agar culture at ordinary room temperature. They were removed from the mating bottles three or four days before the new flies were expected to hatch and no flies were counted after the tenth day from hatching, since ten days is the length of the normal life cycle. In this way the possibility of including flies of a succeeding generation was obviated.

ORIGIN OF "BEAN":

D. melanogaster, as it occurs in nature, has an almost round eye with a facet number of over seven hundred if reared at 25°C, although the number varies with the temperature. The eye of the mutant known as "Bar" is a narrow band with a facet number of about one hundred. When a bar-eyed female is crossed with a full-eyed male the F_1 generation consists of female flies with kidney-shaped eyes and bar-eyed males (see fig.1), Bar and Full being sex-linked characters. However, at the time of

a - Full

b - Bar

c - Heterozygous ♀

d - Double-bar

e - Bean

f - Intra-bar

Fig.1 - Different Types of Bar eyes.

making this cross I was under the impression that Bar was completely dominant over Full, as stated in the various elementary text-books at my disposal, and had expected all the flies to be bar-eyed. I, therefore, brought the females with the kidney-shaped eyes to the attention of Prof. Roberts who, thinking that it might be a mutation, took the matter up with Dr. Morgan.

During the summer I was in charge of the *Drosophila* stocks and it was my duty to obtain pure stocks of bar- and full-eyed flies from the flies which had been used for class work. This necessitated careful examination of the hybrid flies and it was while thus engaged that I observed a male fly with an eye which resembled to some extent the kidney-shaped eye of the females already referred to. The eye of this male was smaller than that of the female but it had a slight nick in its anterior margin. Just about this time Prof. Roberts received a reply from Dr. Morgan with the request that some of the kidney-eyed flies be sent him for examination. These flies were accordingly sent to him, together with the male referred to above and his reply follows:

> "The "kidneys" are only the heterozygous females of "Bar". If you have also obtained a broad Bar male it must be one of the other mutations of Bar."

This was the first intimation that Bar was not fully dominant over Full and that the kidney-shaped eye was therefore only the hybrid condition and could not breed true. However, there remained the broad-barred male with the nick in its anterior margin, which could not be due to a heterozygous condition since the male has only one X-chromosome. Subsequently, more of these peculiar males were obtained and comparison with diagrams of the various bar mutations showed that it differed from any of these. (See fig.1).

MUTATIONS:

The term "mutant" is applied to an individual which varies from the wild type in any character provided the variation, which is known as a mutation, is of a genetic nature, i.e. not due to some external force. The wild D. melanogaster, as stated above, has full red eyes and the first mutant, having white eyes instead of the normal red eyes, appeared in 1910, and, while there was no indication that this white-eyed mutant owed its origin to any peculiarity of the environment, there was, on the other hand, no proof that it had not arisen as the result of some environmental factor. However, as yet there is no evidence to show that there is any relation between a specific external influence and a particular mutation. How or why mutations arise is, therefore, not known.

There are definite types of mutations, depending on whether they involve whole chromosomes, sections of chromosomes or merely a single locus. In the latter case they are termed point mutations. The other types result from such phenomena as non-disjunction, triploidy, tetraploidy, and deficiency and duplication, but by far the greater number of mutations have arisen as the result of a change in a single locus. Some four hundred mutations are now known in the fruit fly and they fall into four linkage groups, coinciding with the four pairs of chromosomes.

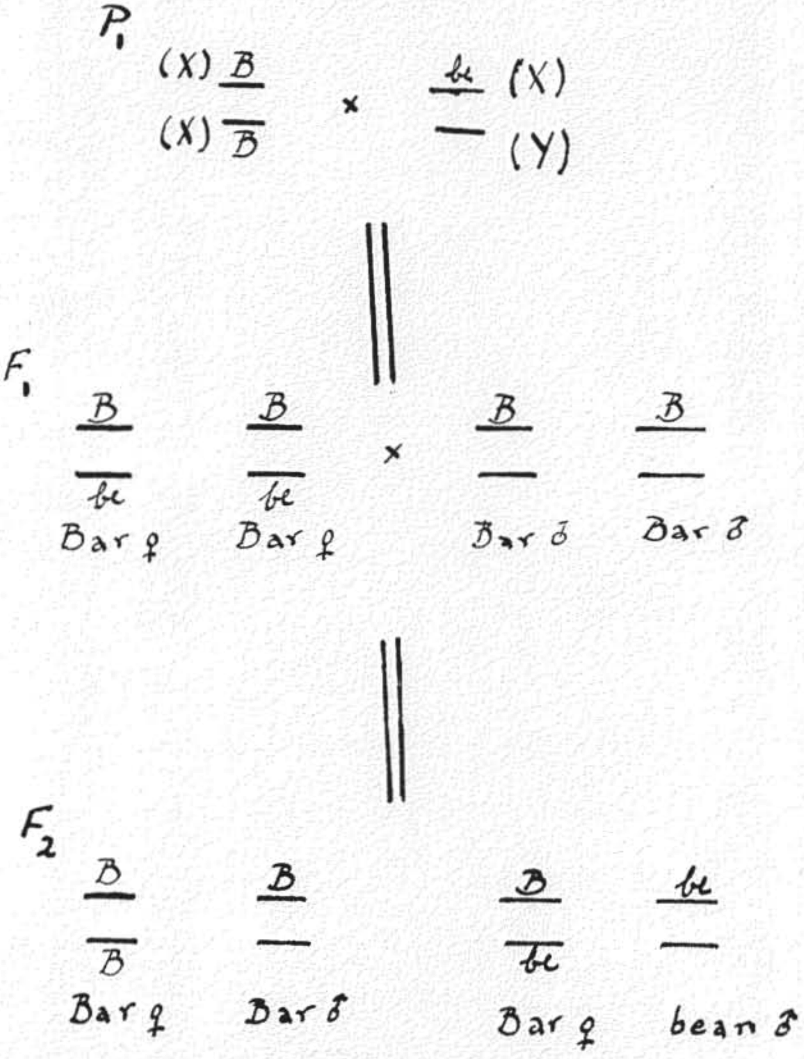
A point of interest in connection with point or gene mutations is that certain loci have mutated repeatedly while others have not been observed to mutate more than once. Among these more mutable genes is that for Full eye which, so far, has only given one mutation, viz. Bar eye, which was discovered by Tice in 1914 when he found a single male with narrow eyes. Bar itself, however, has proved to be one of the most mutable of genes. In 1917, May reported that the Bar gene occasionally reverts to normal. Zeleny, in 1920, found that homozygous Bar gave rise to a new and more extreme allelomorph of Bar which he called ultra-bar, the facet number of which is only 25 at 25°C. This latter mutation was also found to revert to Full as well as to Bar. In 1923, Sturtevant and Morgan put forth the hypothesis that both reversion and the production of double-bar (ultra-bar of Zeleny) are due to unequal crossing-over at the Bar locus. A further mutation of the Bar gene known as infra-bar, with a facet number of 320.4

at 25°C, unlike the other mutations of the Bar gene which have been recorded is not due to unequal crossing-over but represents a modification of the Bar gene itself.

THE MUTATION "BEAN" - PRELIMINARY CROSSES:

The mutation to be discussed here has been given the name of "bean" and was first observed in the summer of 1925 as already described but it was not until the fall of the same year that the problem of attempting to find to which linkage group this character belonged was taken up. Since the mutation had appeared only in male individuals the natural assumption was of course that it was a sex-linked character and that if it were crossed with a Bar female it would reappear in twenty-five per cent. of the individuals in the F_2 generation as shown in Fig.2. These bean-eyed individuals should, however, all be males. All the females should be bar-eyed but only half the males should be bar-eyed, the remainder being bean-eyed.

Bar ♀ × bean ♂



3 Bar : 1 Bean.

Fig. 2 - Inheritance of a Sex-linked character.

In order to test the validity of this assumption the following crosses were made:-

Cross No.1

Bar ♀ x Bean ♂

F_1 Generation

Bar	Bean
35	

F_2 Generation

Bar	Bean
73	5 (♂'s)

Ratio of Bar to bean - 14:1

Cross No.2

Bar ♀ x Bean ♂

F_1 Generation

Bar	Bean
25	

F_2 Generation

Bar	Bean
156	22 (♂'s)

Ratio of Bar to bean - 7:1

Cross No.3

Bar ♀ x Bean ♂

F_1 Generation

Bar	Bean
33	4

F_2 Generation

Bar	Bean
289	44 (♂'s)

Ratio of Bar to bean - 6:1

Cross No.4

Bar♀ x Bean♂

F_1 Generation

Bar	Bean
31	

F_2 Generation

Bar	Bean
184	20 (♂'s)

Ratio of Bar to bean - 9:1

Cross No.5

Bar♀ x Bean♂

F_1 Generation

Bar	Bean
21	

F_2 Generation

Bar	Bean
87	

These crosses, excluding No.5, give a total of 702 Bar-eyed flies to 91 bean-eyed flies, a ratio of, roughly, 7:1.

Certain discrepancies are apparent in the results of these crosses and can be discussed under the following heads:

1. Non-appearance of bean in F_2 Generation of Cross No.5.

No bean-eyed males were obtained in the F_2 generation of this cross. Since the parents had not been preserved it was impossible to ascertain whether the male parent had really possessed the mutation. A Bar male might have been used accidentally.

2. Percentage of bean-eyed flies in F_2 Generation.

On the assumption that "bean" was a sex-linked recessive, twenty-five per cent. of the F_2 generation, representing one-half of the total male population, should have been bean-eyed. In the four crosses in which "bean" appeared in the F_2 offspring the number of bean-eyed individuals was too small. The total F_2 offspring, i.e. from the four crosses, was not really large enough to warrant any definite conclusion but the fact that no bean-eyed females appeared as well as the fact that the bean character did reappear in some of the F_2 males seemed to confirm the original assumption that "bean" was a sex-linked recessive mutation.

3. Appearance of bean-eyed flies in F_1 of Cross No.3.

Unfortunately the flies from these crosses were not preserved and, therefore, it is not possible to re-examine them. Sometimes the bar eye shows a slight defect in its anterior margin, thus resembling the "bean" eye, and some such flies may have been classified wrongly as bean-eyed. The "bean" eye, however, in addition to the defect in its margin is wider than the bar eye and of a slightly different shape. (see Fig.1,e.).

These crosses did seem to indicate, however, that the mutation was of a genetic nature and it was consequently deemed advisable to attempt to establish a stock of bean-eyed flies before proceeding with further crosses, for thus far no bean-eyed female had appeared among the offspring of the crosses made. It was hoped to accomplish this by back-crossing the F_1 hybrid female of a bar-bean cross to a bean-eyed male, for reference to Fig.2 will show that if the mutation were a sex-linked recessive the F_1 hybrid females would carry the gene for "bean" in one X-chromosome and Bar in the other X-chromosome. If this heterozygous female were mated to a bean-eyed male half the females from this cross should have "bean" eyes since, as Fig.3 shows, both the X-chromosomes would carry the gene for "bean" eye.

Bar ♀ × bean ♂

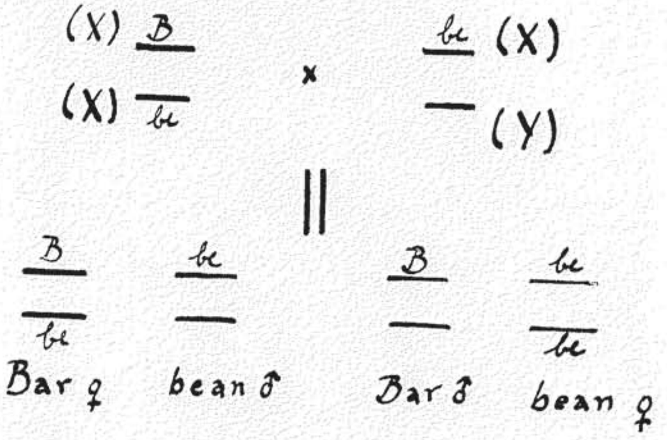


Fig. 3 - Backcross, Hybrid ♀ × bean ♂.

A number of these crosses were made but, contrary to expectation, no bean-eyed females were obtained.

At about this time I came upon Dr. Charles Zeleny's paper entitled "Germinal Changes in the Bar-Eyed Race of Drosophila during the Course of Selection for Facet Number", in which he says:

> "In the high facet selection line marked mutations have occurred which have yielded full-eyed individuals indistinguishable from the wild ones which originally mutated to form the bar stock. These new full-eyed flies are genetically of two distinct types. One type is the result of a reverse mutation involving the return of the bar gene to the original full-eye-producing condition. Its hereditary behavior is similar to that of the wild Drosophila in all the tests that have been made.
>
> "The other type retains the bar gene unchanged, the somatic appearance of full eye being due to the formation

> of a modifying gene outside of the sex chromosome. This new gene is effective in producing full eye when present in double dose in females heterozygous for the bar gene. Such full-eyed females when crossed with wild full-eyed males produce males half of whom are bar and females half of whom are heterozygous bar.
>
> "In males with the bar gene and in females homozygous for bar the double dose of the new gene produces an eye which is nearly full but which differs from full in the presence of a defect at the anterior margin. Such an eye may be designated by the term "emarginate". Emarginate females when crossed with full wild males give males all of whom are bar and females all of whom are heterozygous bar. Numerous tests bear out in detail the hypothesis as stated above indicating that the chromosomal formula for this type of female with a full eye is $\frac{B}{} \ \frac{m}{m}$, for the emarginate-eyed female $\frac{B}{B} \ \frac{m}{m}$, and for the emarginate-eyed male $\frac{B}{} \ \frac{m}{m}$ Experiments are under way to determine the exact locus of the new gene."

The description of Dr. Zeleny's "emarginate" eye suggested the somatic appearance of "bean". Also, "emarginate" first appeared in a male individual but Dr. Zeleny was able, after some difficulty, to obtain the female. Prof. Roberts accordingly communicated with Dr. Zeleny with a view to ascertaining whether the two mutations were the same. An account of the crosses which had been made were of course sent to Dr. Zeleny, who replied that "bean" was probably a different mutation from "emarginate".

It was impossible to continue further with the problem at the University of Manitoba because Prof. Roberts had not the stocks necessary for further tests. Consequently, in May 1926 I took the bar stock, from which the mutant specimens had arisen, to Dr. Zeleny's laboratory at the University of Illinois, but before discussing the crosses which were then made it seems advisable to digress from the experimental work for a short time to discuss the cytological aspect of the problem.

CYTOLOGICAL ASPECT OF PROBLEM:

D. melanogaster has four pairs of chromosomes. Of these one pair is known as the sex-chromosomes. Of this pair, in the cells of the female the two chromosomes have the same form while in the cells of the male they differ, one having a small hook. The second pair of chromosomes is the longest, the third pair slightly shorter, while the fourth pair is very short. The chromosomes, as they appear in the male and female cells, are shown in Fig.4. After maturation the ripe ova contain one sex-chromosome, or X-chromosome, as it is usually termed, plus three autosomes, as the other chromosomes are termed, while the spermatozoa may contain either an X-chromosome or a Y-chromosome, as the sex-chromosome with the hook is designated, plus the three autosomes. Each chro-

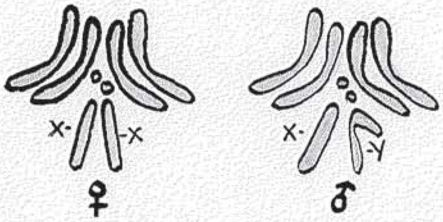


Fig 4 - Chromosomes of D. melanogaster.

mosome, at certain stages in the maturation divisions, can be seen to be made up of smaller portions strung together like a string of beads, but this appearance of the chromosomes is lost in the mature germ cells. The chromosomes are believed to maintain their identity throughout the resting stages of the nucleus although the cytological evidence on this point is not as yet conclusive. From a genetic standpoint, however, this is a fundamental requirement since each chromosome is believed to be made up of a definite number of factors or genes, each with its own locus. This idea of the chromosome has given rise to the somewhat erroneous conception of unit characters, i.e. that each locus is the seat of a given character. However, the concensus of opinion suggests that any given character may represent either the end-product of the interaction of many genes or possibly all the genes. In this process certain changes in one of the genes, all the others remaining quite unchanged, bring about a change in the end-result, giving rise in turn to the mutation. For this reason the locus responsible for the change in the end-result has been given the name of the mutation.

The determination of the locus of each gene in the chromosomes of Drosophila has been the work, to a very great extent, of Prof. T. H. Morgan and his students. This is determined by crossing-over experiments. While crossing-over has not been confirmed cytologically it is, one might say, a genetic fact. If no crossing-over were to take place then genes in the same chromosome entering a cross together should come out of the cross in the same way. This idea is known as linkage. However, these genes do not always remain linked, as was discovered early on in the work by Bateson and Punnett in sweet peas. Briefly, the percentage of crossing-over is a measure of the distance between to given genes, i.e. if two genes are close together the chances of a break occurring between them are much smaller than if they are widely separated by intervening genes. As a result of numerous crossing-over experiments it has been possible to plot what are known as "chromosome maps", that for D. melanogaster being shown in Fig.5.

DETERMINATION OF LINKAGE GROUP OF A MUTATION:

In order to determine to which linkage group a new mutant belongs it is crossed with specially made up stocks, each of which carries a number of recessive characters of one of the linkage groups. These stocks are known as the X-ple, II-ple, III-ple and IV-ple stocks. However, instead of using the X-ple stock it is much simpler to test for a sex-linked character by crossing a mutant male with what is known as a Double Yellow Non-disjunction (YY) female. The females of this stock have their two X-chromosomes permanently united at one end, as shown in fig.6, as a result of which at the

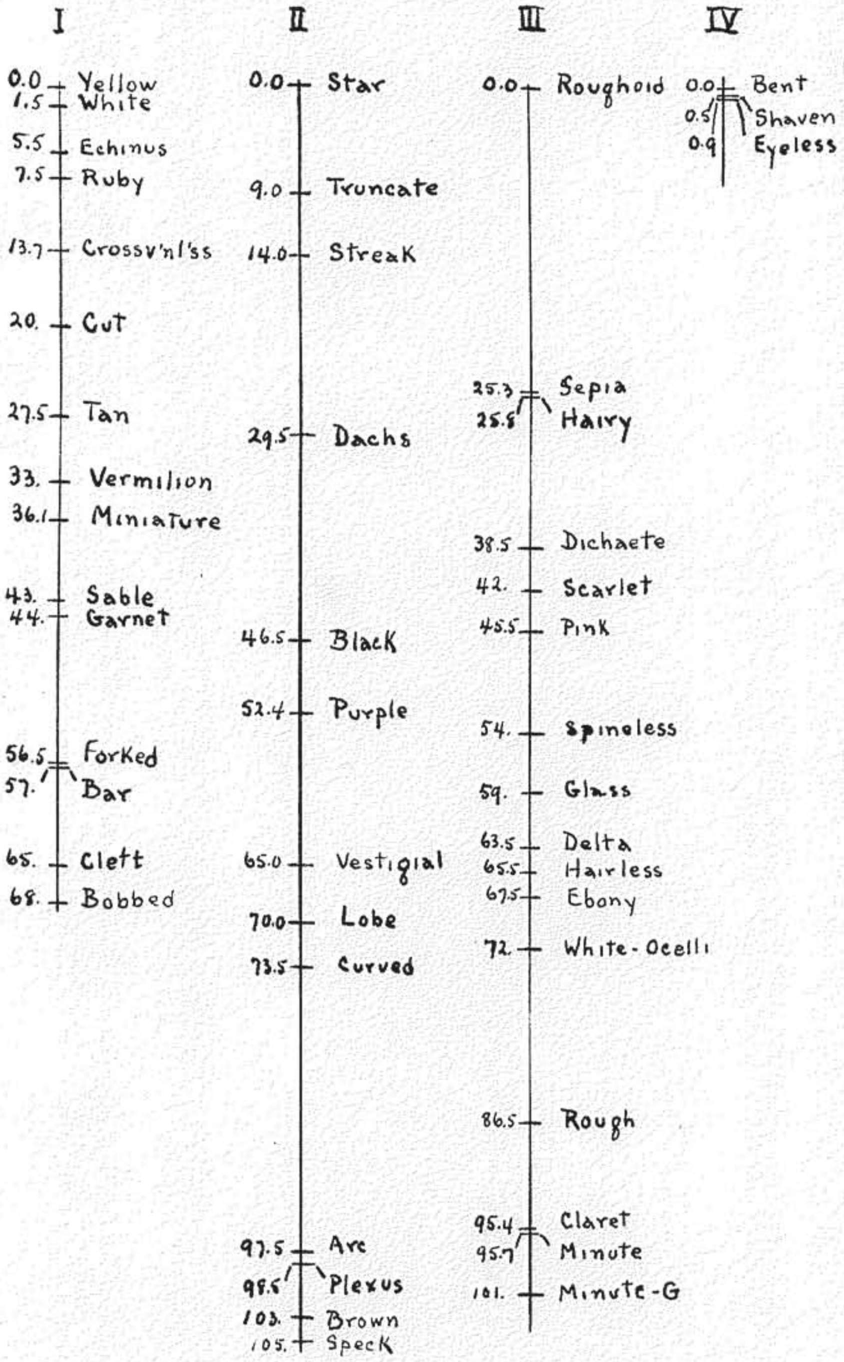


Fig 5 - Chromosome map of *D. melanogaster.*

reduction division either polar body or ovum is left without an X-chromosome. Consequently, the males receive their X-chromosome from the male parent. In ordinary sex-linked inheritance the sons inherit their sex-linked characters from the mother since they get their single X-chromosome from her, but the reverse of course takes place when the non-disjunction stock is used - the sons now inherit their sex-linked characters from the father since they get their X-chromosome from him. Therefore, if a mutation is sex-linked the F_1 males of a cross between a non-disjunction female and the mutant male should show the mutation. Fig.7 illustrates such a cross.

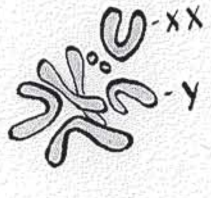


Fig 6 - Chromosomes of Non-disjunction ♀.

The II-ple (Second chromosome complex) stock carries the following recessive characters:

1. black (b) - body colour black, veins black bordered.
2. purple (pr) - purplish-pink eye-colour.
3. curved (c) - wings concave below, thin-textured, divergent, up-lifted.

Non-disjunction ♀ × bean ♂.

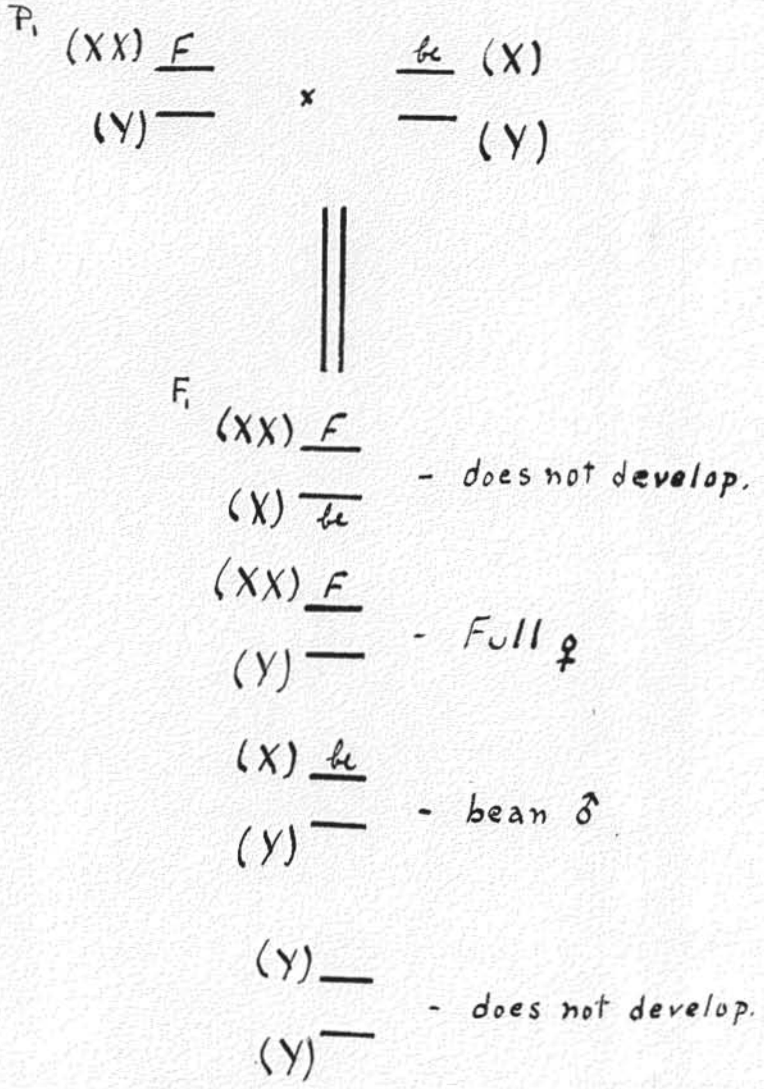


Fig 7 - Sex-linked Inheritance with Non-disjunction stock.

and a mutation is a member of the second linkage group if it shows linkage to "black" or some other mutant already known to be linked to "black".

The III-ple (Third chromosome complex) stock carries the following recessive characters:

1. scarlet (st) - vermilion eye-colour, opaque, with age becomes darker than vermilion or cinnabar.
2. spineless (ss) - bristles very short.
3. sooty (e^s) - an allelomorph of ebony but not so dark.
4. roughoid (ru) - eye slighly smaller, rougher disarrangement of facets. Slight smudge of blackened facets in lower part of eye.
5. hairy (h) - extra hairs along veins of wings, on scutellum, on head and on mesopleura.

and a mutation that shows linkage to "pink" or some other mutation already known to be linked to "pink" is a member of the third linkage group.

The fourth chromosome is very small morphologically and the number of genes known for it at present is only three, viz. bent, shaven and eyeless. As in the other cases, if a mutation shows linkage to any of these characters it is naturally a member of the fourth linkage group.

Crossing-over does not occur in the male and this fact makes it very simple to determine to which group an autosomal mutation belongs for a new, non-sex-linked recessive will give in the F_2 generation a 2:1:1:0 ratio with any other recessives of the group to which it belongs. An example may make this clearer. A certain mutant arose which was called spineless and proved to be recessive. When a spineless fly was crossed to a fly with pink eyes the F_2 generation resolved itself into 2 wild-type: 1 pink: 1 spineless: 0 pink spineless, a ratio which showed that spineless is linked to pink. When, on the other hand, spineless was crossed to a black mutant, black belonging to the second group, the F_2 generation gave the following ratio - 9 wild-type: 3 black: 3 spineless: 1 black spineless, which ratio indicates free assortment of the chromosomes and means that spineless does not belong to the same linkage group as black. Figures 8 and 9 illustrate this.

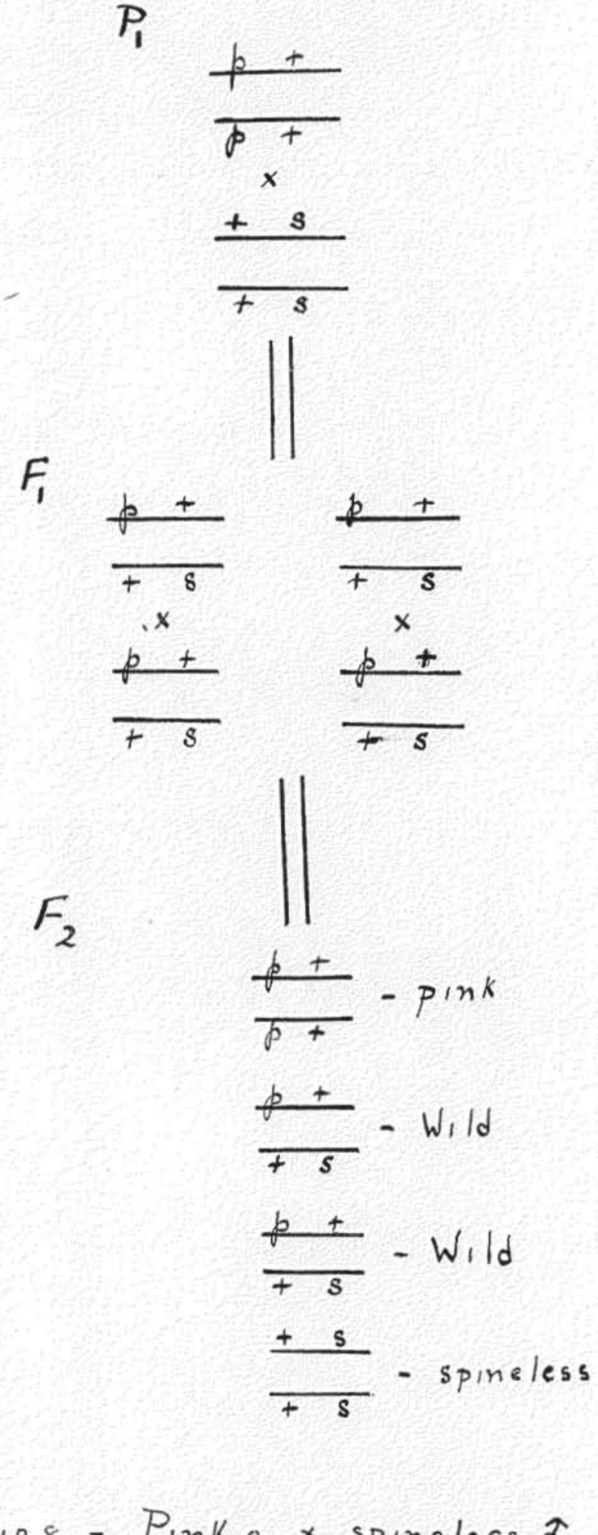


Fig 8 - Pink ♀ × spineless ♂.

Spineless ♀ x black ♂

P₁

$$\frac{s}{s}\ \frac{+}{+}$$

x

$$\frac{+}{+}\ \frac{b}{b}$$

$$\Big\|$$

F₁

$$\frac{s}{+}\ \frac{+}{b} \qquad \frac{s}{+}\ \frac{+}{b}$$

x x

$$\frac{s}{+}\ \frac{+}{b} \qquad \frac{s}{+}\ \frac{+}{b}$$

$$\Big\|$$

F₂

$\frac{s}{s}\ \frac{+}{+}$ - spineless

$\frac{s}{s}\ \frac{+}{b}$ - spineless

$\frac{s}{+}\ \frac{+}{b}$ - Wild

$\frac{s}{+}\ \frac{+}{+}$ - Wild

$\frac{s}{s}\ \frac{b}{+}$ - spineless

$\frac{s}{s}\ \frac{b}{b}$ - black spineless

$\frac{s}{+}\ \frac{b}{b}$ - black

$\frac{s}{+}\ \frac{b}{+}$ - Wild

$\frac{+}{s}\ \frac{b}{+}$ - Wild

$\frac{+}{s}\ \frac{b}{b}$ - black

$\frac{+}{+}\ \frac{b}{b}$ - black

$\frac{+}{+}\ \frac{b}{+}$ - Wild

$\frac{+}{s}\ \frac{+}{+}$ - Wild

$\frac{+}{s}\ \frac{+}{b}$ - Wild

$\frac{+}{+}\ \frac{+}{b}$ - Wild

$\frac{+}{+}\ \frac{+}{+}$ - Wild

Fig 9 - Inheritance of 2 factors in different chromosomes.

Since the behaviour of "bean" pointed to its being a sex-linked recessive the first cross made was between it and a double-yellow permanent non-disjunction female with full eyes. From a cross of this sort then (i.e. non-disjunction female x bean male) the sons should all show the "bean" character if this gene is a member of the first linkage group. Six such crosses were made, with the following results:

females - full eye
males - bar eye

In all 368 flies were examined but no bean-eyed flies were obtained. This was conclusive proof that the mutation was not a member of the first linkage group. However, since the males from this cross were bar-eyed, it further proved that the bar gene must be carried in the X-chromosome of the bean-eyed individual and that such an individual must carry a gene in one of its autosomes which acts as a modifier of the bar gene. The problem thus narrowed itself down to whether this modifier was a member of the second, third or fourth linkage groups.

In order to determine whether "bean" was a member of the second linkage group a bean-eyed male was crossed with a II-ple female and the results of these crosses are listed below:

	F_1		F_2				
	Het. ♀	Full ♂	Het. ♀	Full ♀	Full ♂	Bar ♂	bean ♂
1.	24	44	209	218	238	134	37
2.	43	42	195	198	209	154	31
3.	32	26	197	196	184	149	26
4.	66	54	353	382	360	262	61
5.	29	28	242	268	257	135	56
6.	45	43	261	241	273	176	45
7.	37	32	192	231	207	135	48
8.	37	29	117	84	89	44	43
9.	40	32	128	152	149	74	66
10.	53	36	149	171	153	72	47

Since a number of the bean-eyed individuals carried one or more of the II-ple characters it was thus proved that the bean modifier could not be a member of the second linkage group. Fig.10 represents diagramatically the chromosomal formulae of the classes from this cross assuming that the bean gene modifier is in either chromosome III or IV.

II-ple ♀ × bean ♂

P_1

$$\frac{F}{F}\quad\frac{b\ pr\ c}{b\ pr\ c}\quad\frac{+}{+}\quad\frac{+}{+}$$

×

$$\frac{B}{—}\quad\frac{+\ +\ +}{+\ +\ +}\quad\frac{be}{be}\quad\frac{+}{+}$$

$$\|$$

F_1

$$\frac{F}{B}\quad\frac{b\ pr\ c}{+\ +\ +}\quad\frac{+}{be}\quad\frac{+}{+}$$ – Heterozygous ♀

×

$$\frac{F}{—}\quad\frac{b\ pr\ c}{+\ +\ +}\quad\frac{+}{be}\quad\frac{+}{+}$$ – Full ♂

$$\|$$

F_2

$$\frac{F}{F}\quad\frac{b\ pr\ c}{b\ pr\ c}\quad\frac{+}{+}\quad\frac{+}{+}$$ – Full ♀

$$\frac{F}{F}\quad\frac{b\ pr\ c}{b\ pr\ c}\quad\frac{+}{be}\quad\frac{+}{+}$$ – Full ♀

$$\frac{F}{F}\quad\frac{b\ pr\ c}{+\ +\ +}\quad\frac{+}{+}\quad\frac{+}{+}$$ – Full ♀

$$\frac{F}{F}\quad\frac{b\ pr\ c}{+\ +\ +}\quad\frac{+}{be}\quad\frac{+}{+}$$ – Full ♀

$\frac{F}{\text{—}}\ \frac{b\ pr\ c}{+\ +\ +}\ \frac{+}{bc}\ \frac{+}{+}$ - Full ♂

$\frac{F}{\text{—}}\ \frac{b\ pr\ c}{+\ +\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{\text{—}}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{+}{bc}\ \frac{+}{+}$ - Full ♂

$\frac{F}{\text{—}}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{F}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{bc}{+}\ \frac{+}{+}$ - Full ♀

1. $\frac{F}{F}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{bc}{bc}\ \frac{+}{+}$ - Full ♀

$\frac{F}{F}\ \frac{b\ pr\ c}{+\ +\ +}\ \frac{bc}{+}\ \frac{+}{+}$ - Full ♀

2. $\frac{F}{F}\ \frac{b\ pr\ c}{+\ +\ +}\ \frac{bc}{bc}\ \frac{+}{+}$ - Full ♀

3. $\frac{F}{\text{—}}\ \frac{b\ pr\ c}{+\ +\ +}\ \frac{bc}{bc}\ \frac{+}{+}$ - Full ♂

$\frac{F}{\text{—}}\ \frac{b\ pr\ c}{+\ +\ +}\ \frac{bc}{+}\ \frac{+}{+}$ - Full ♂

4. $\frac{F}{-}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{be}{be}\ \frac{+}{+}$ - Full ♂

$\frac{F}{-}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{be}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{F}\ \frac{+\ +\ +}{b\ pr\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♀

$\frac{F}{F}\ \frac{+\ +\ +}{b\ pr\ c}\ \frac{+}{be}\ \frac{+}{+}$ - Full ♀

$\frac{F}{F}\ \frac{+\ +\ +}{+\ +\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♀

$\frac{F}{F}\ \frac{+\ +\ +}{+\ +\ +}\ \frac{+}{be}\ \frac{+}{+}$ - Full ♀

$\frac{F}{-}\ \frac{+\ +\ +}{+\ +\ +}\ \frac{+}{be}\ \frac{+}{+}$ - Full ♂

$\frac{F}{-}\ \frac{+\ +\ +}{+\ +\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{-}\ \frac{+\ +\ +}{b\ pr\ c}\ \frac{+}{be}\ \frac{+}{+}$ - Full ♂

$\frac{F}{-}\ \frac{+\ +\ +}{b\ pr\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{F}\ \frac{+\,+\,+}{b\ pr\ c}\ \frac{be}{+}\ \frac{+}{+}$ - Full ♀

5. $\frac{F}{F}\ \frac{+\,+\,+}{b\ pr\ c}\ \frac{be}{be}\ \frac{+}{+}$ - Full ♀

$\frac{F}{F}\ \frac{+\,+\,+}{+\,+\,+}\ \frac{be}{+}\ \frac{+}{+}$ - Full ♀

6. $\frac{F}{F}\ \frac{+\,+\,+}{+\,+\,+}\ \frac{be}{be}\ \frac{+}{+}$ - Full ♀

7. $\frac{F}{\quad}\ \frac{+\,+\,+}{+\,+\,+}\ \frac{be}{be}\ \frac{+}{+}$ - Full ♂

$\frac{F}{\quad}\ \frac{+\,+\,+}{+\,+\,+}\ \frac{be}{+}\ \frac{+}{+}$ - Full ♂

8. $\frac{F}{\quad}\ \frac{+\,+\,+}{b\ pr\ c}\ \frac{be}{be}\ \frac{+}{+}$ - Full ♂

$\frac{F}{\quad}\ \frac{+\,+\,+}{b\ pr\ c}\ \frac{be}{+}\ \frac{+}{+}$ - Full ♂

$\frac{B}{F}\ \frac{+\,+\,+}{b\ pr\ c}\ \frac{be}{+}\ \frac{+}{+}$ - Heterozygous ♀

9. $\frac{B}{F}\ \frac{+\,+\,+}{b\ pr\ c}\ \frac{be}{be}\ \frac{+}{+}$ - Het. ♀

$\frac{\mathrm{B}}{-}\;\frac{+\,+\,+}{\mathrm{b\ pr\ c}}\;\frac{+}{+}\;\frac{+}{+}$ - Bar ♂

$\frac{\mathrm{B}}{-}\;\frac{+\,+\,+}{\mathrm{b\ pr\ c}}\;\frac{+}{\mathrm{be}}\;\frac{+}{+}$ - Bar ♂

$\frac{\mathrm{B}}{-}\;\frac{+\,+\,+}{+\,+\,+}\;\frac{+}{\mathrm{be}}\;\frac{+}{+}$ - Bar ♂

$\frac{\mathrm{B}}{-}\;\frac{+\,+\,+}{+\,+\,+}\;\frac{+}{+}\;\frac{+}{+}$ - Bar ♂

$\frac{\mathrm{B}}{\mathrm{F}}\;\frac{\mathrm{b\ pr\ c}}{\mathrm{b\ pr\ c}}\;\frac{\mathrm{be}}{+}\;\frac{+}{+}$ - Het. ♀

13. $\frac{\mathrm{B}}{\mathrm{F}}\;\frac{\mathrm{b\ pr\ c}}{\mathrm{b\ pr\ c}}\;\frac{\mathrm{be}}{\mathrm{be}}\;\frac{+}{+}$ - Het. ♀

14. $\frac{\mathrm{B}}{\mathrm{F}}\;\frac{\mathrm{b\ pr\ c}}{+\,+\,+}\;\frac{\mathrm{be}}{\mathrm{be}}\;\frac{+}{+}$ - Het. ♀

$\frac{\mathrm{B}}{\mathrm{F}}\;\frac{\mathrm{b\ pr\ c}}{+\,+\,+}\;\frac{\mathrm{be}}{+}\;\frac{+}{+}$ - Het. ♀

15. $\frac{\mathrm{B}}{-}\;\frac{\mathrm{b\ pr\ c}}{+\,+\,+}\;\frac{\mathrm{be}}{\mathrm{be}}\;\frac{+}{+}$ - Bean ♂ (with wild characters)

$\frac{\mathrm{B}}{-}\;\frac{\mathrm{b\ pr\ c}}{+\,+\,+}\;\frac{\mathrm{be}}{+}\;\frac{+}{+}$ - Bar ♂

$\frac{B}{—}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{be}{be}\ \frac{+}{+}$ - bean ♂ (with II-ple characters).

$\frac{B}{—}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{be}{+}\ \frac{+}{+}$ - Bar ♂

$\frac{B}{F}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{F}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{+}{be}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{F}\ \frac{b\ pr\ c}{+\ +\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{F}\ \frac{b\ pr\ c}{+\ +\ +}\ \frac{+}{be}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{—}\ \frac{b\ pr\ c}{+\ +\ +}\ \frac{+}{be}\ \frac{+}{+}$ - Bar ♂

$\frac{B}{—}\ \frac{b\ pr\ c}{+\ +\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Bar ♂

$\frac{B}{—}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{+}{be}\ \frac{+}{+}$ - Bar ♂

$\frac{B}{—}\ \frac{b\ pr\ c}{b\ pr\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Bar ♂

Fig. 10-Inheritance of bean, assuming bean is in Chromosome III.

$\frac{B}{F}\;\frac{+\,+\,+}{+\,+\,+}\;\frac{be}{+}\;\frac{+}{+}$ - Het. ♀

10. $\frac{B}{F}\;\frac{+\,+\,+}{+\,+\,+}\;\frac{be}{be}\;\frac{+}{+}$ - Het. ♀

11. $\frac{B}{-}\;\frac{+\,+\,+}{+\,+\,+}\;\frac{be}{be}\;\frac{+}{+}$ - bean ♂ (with wild characters)

$\frac{B}{-}\;\frac{+\,+\,+}{+\,+\,+}\;\frac{be}{+}\;\frac{+}{+}$ - Bar ♂

12. $\frac{B}{-}\;\frac{+\,+\,+}{b\ pr\ c}\;\frac{be}{be}\;\frac{+}{+}$ - Bean ♂ (with wild characters)

$\frac{B}{-}\;\frac{+\,+\,+}{b\ pr\ c}\;\frac{be}{+}\;\frac{+}{+}$ - Bar ♂

$\frac{B}{F}\;\frac{+\,+\,+}{b\ pr\ c}\;\frac{+}{+}\;\frac{+}{+}$ - Heterozygous ♀

$\frac{B}{F}\;\frac{+\,+\,+}{b\ pr\ c}\;\frac{+}{be}\;\frac{+}{+}$ - Het. ♀

$\frac{B}{F}\;\frac{+\,+\,+}{+\,+\,+}\;\frac{+}{+}\;\frac{+}{+}$ - Het. ♀

$\frac{B}{F}\;\frac{+\,+\,+}{+\,+\,+}\;\frac{+}{be}\;\frac{+}{+}$ - Het. ♀

Fig.11 simply shows the classes to be expected from a II-ple x bean-eyed male cross, assuming that the bean modifier is a member of the second linkage group. None of the bean-eyed individuals would carry any of the II-ple characters and since such individuals were obtained the gene for the bean modifier was eliminated from the second chromosome.

Crossing-over does of course take place in the female but nevertheless the bean-eyed flies from the above cross would not show any of the recessive characters because when they are present in the chromosome derived from the female they will be masked over by the wild allelomorphs in the homologous chromosome from the male, as fig.12 shows.

II-ple ♀ × bean ♂

P_1

$$\frac{F}{F}\ \frac{b\ pr + c}{b\ pr + c}\ \frac{+}{+}\ \frac{+}{+}$$

×

$$\frac{B}{\quad}\ \frac{+ + bc +}{+ + bc +}\ \frac{+}{+}\ \frac{+}{+}$$

$$\Bigg\|$$

F_1

$$\frac{F}{B}\ \frac{b\ pr + c}{+ + bc +}\ \frac{+}{+}\ \frac{+}{+}$$ - Heterozygous ♀

×

$$\frac{F}{\quad}\ \frac{b\ pr + c}{+ + bc +}\ \frac{+}{+}\ \frac{+}{+}$$ - Full ♂

$$\Bigg\|$$

F_2

$$\frac{F}{F}\ \frac{b\ pr + c}{b\ pr + c}\ \frac{+}{+}\ \frac{+}{+}$$ - Full ♀

$$\frac{F}{F}\ \frac{b\ pr + c}{+ + bc +}\ \frac{+}{+}\ \frac{+}{+}$$ - Full ♀

$\frac{F}{-}\ \frac{b\ pr + c}{b\ pr + c}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{-}\ \frac{b\ pr + c}{+\ +\ be\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{F}\ \frac{+\ +\ be\ +}{b\ pr + c}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♀

$\frac{F}{F}\ \frac{+\ +\ be\ +}{+\ +\ be\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♀

$\frac{F}{-}\ \frac{+\ +\ be\ +}{b\ pr + c}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{-}\ \frac{+\ +\ be\ +}{+\ +\ be\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{B}{F}\ \frac{b\ pr + c}{b\ pr + c}\ \frac{+}{+}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{F}\ \frac{b\ pr + c}{+\ +\ be\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{-}\ \frac{b\ pr + c}{b\ pr + c}\ \frac{+}{+}\ \frac{+}{+}$ - Bar ♂

$\frac{B}{-}\ \frac{b\ pr + c}{+\ +\ be\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Bar ♂

$\frac{B}{F}\ \frac{+\ +\ be\ +}{b\ pr\ +\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{F}\ \frac{+\ +\ be\ +}{+\ +\ be\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{-}\ \frac{+\ +\ be\ +}{b\ pr\ +\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Bar ♂

$\frac{B}{-}\ \frac{+\ +\ be\ +}{+\ +\ be\ +}\ \frac{+}{+}\ \frac{+}{+}$ - bean ♂ (with wild characters)

Fig 11 - Inheritance of bean, assuming bean is in Chromosome II.

Heterozygous ♀ × Full ♂ (F₁'s of previous cross).

Formulae of Chromosomes before Crossing-over has taken place.

$$\frac{F}{B}\ \frac{b\ pr + c}{+ + be +}\ \frac{+}{+}\ \frac{+}{+}$$

$$\times$$

$$\frac{F}{—}\ \frac{b\ pr + c}{+ + be +}\ \frac{+}{+}\ \frac{+}{+}$$

It is assumed that the break in the chromosomes takes place between "pr" and "+".

Formulae of Chromosomes after Crossing-over has taken place.

$$\frac{F}{B}\ \frac{b\ pr\ be +}{+ + + c}\ \frac{+}{+}\ \frac{+}{+}$$

$$\times$$

$$\frac{F}{—}\ \frac{b\ pr + c}{+ + be +}\ \frac{+}{+}\ \frac{+}{+}$$

‖

$$\frac{F}{F}\ \frac{b\ pr\ be +}{b\ pr + c}\ \frac{+}{+}\ \frac{+}{+}$$ - Full ♀

$$\frac{F}{F}\ \frac{b\ pr\ be +}{+ + be +}\ \frac{+}{+}\ \frac{+}{+}$$ - Full ♀

$\frac{F}{-}\ \frac{b\ pr\ bc\ +}{+\ +\ bc\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{-}\ \frac{b\ pr\ bc\ +}{b\ pr\ +\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{F}\ \frac{+\ +\ +\ c}{b\ pr\ +\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♀

$\frac{F}{F}\ \frac{+\ +\ +\ c}{+\ +\ bc\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♀

$\frac{F}{-}\ \frac{+\ +\ +\ c}{+\ +\ bc\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{F}{-}\ \frac{+\ +\ +\ c}{b\ pr\ +\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Full ♂

$\frac{B}{F}\ \frac{+\ +\ +\ c}{b\ pr\ +\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{F}\ \frac{+\ +\ +\ c}{+\ +\ bc\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{-}\ \frac{+\ +\ +\ c}{+\ +\ bc\ +}\ \frac{+}{+}\ \frac{+}{+}$ - Bar ♂

$\frac{B}{-}\ \frac{+\ +\ +\ c}{b\ pr\ +\ c}\ \frac{+}{+}\ \frac{+}{+}$ - Bar ♂

$\dfrac{B}{F}\ \dfrac{b\ pr\ be\ +}{b\ pr\ +\ c}\ \dfrac{+}{+}\ \dfrac{+}{+}$ - Het. ♀

$\dfrac{B}{F}\ \dfrac{b\ pr\ be\ +}{+\ +\ be\ +}\ \dfrac{+}{+}\ \dfrac{+}{+}$ - Het. ♀

$\dfrac{B}{\quad}\ \dfrac{b\ pr\ be\ +}{+\ +\ be\ +}\ \dfrac{+}{+}\ \dfrac{+}{+}$ - bean ♂ (with wild characters)

$\dfrac{B}{\quad}\ \dfrac{b\ pr\ be\ +}{b\ pr\ +\ c}\ \dfrac{+}{+}\ \dfrac{+}{+}$ - Bar ♂

Fig 12 - Inheritance of bean when crossing-over takes place in ♀.

The bean modifier now being definitely eliminated from the second linkage group a number of crosses were made with the III-ple stock, but the results from these crosses were not at first satisfactory. In the first cross only 5 bean-eyed flies were obtained as against 726 other flies and of these five none carried any III-ple characters. Another cross gave 4 bean-eyed flies as against 399 other flies, while a third gave 1 bean-eyed fly against 897 other flies. This small proportion of bean-eyed flies was very puzzling but it was thought that it might be due to an inhibiting factor for "roughoid", one of the III-ple characters, does make the eye smaller and if the eye were made smaller it might possibly alter its shape at the same time. I, therefore, attempted to obtain a female without "roughoid" but although I obtained individuals which did not show the roughoid appearance they evidently carried the factor as a recessive. In the meantime, however, a further cross with a III-ple female carrying "roughoid" yielded a much higher proportion of bean-eyed individuals, some of which carried III-ple characters. As some of these bean-eyed flies carried "roughoid" I was forced to conclude that in the first crosses this could not have been the only inhibiting fa~~x~~ctor. Following are the results of the III-ple-bean crosses:

	F_1		F_2				
	Het.	Full	Het.	Full		Bar	bean
	♀	♂	♀	♀	♂	♂	♂
1.	41	36	199	189	179	159	5
2.	45	33	98	100	111	90	4
3.	102	112	224	224	227	222	1
4.	105	148	163	170	181	153	2
5.	52	49	172	165	150	152	6
6.	35	44	190	216	212	183	39
7.	84	77	132	130	113	67	17
8.	29	28	80	89	81	56	12

Since some of the bean-eyed individuals carried III-ple characters the bean gene modifier was thus eliminated from the third linkage for if it were a member of this group none of these flies would have carried any of the III-ple characters even when crossing-over takes place in the female, as shown in figures 13 and 14.

III-ple ♀ x bean ♂

$$P_1 \quad \frac{F}{F}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ ss\ e^s}{ru\ h\ st\ +\ ss\ e^s}\ \frac{+}{+}$$

$$\times$$

$$\frac{B}{\text{---}}\ \frac{+}{+}\ \frac{+\ +\ +\ be\ +\ +}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$$

$$F_1 \quad \frac{F}{B}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ ss\ e^s}{+\ +\ +\ be\ +\ +}\ \frac{+}{+} \text{ - Het. ♀}$$

$$\times$$

$$\frac{F}{\text{---}}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ ss\ e^s}{+\ +\ +\ be\ +\ +}\ \frac{+}{+} \text{ - Full ♂}$$

$$F_2 \quad \frac{F}{F}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ ss\ e^s}{ru\ h\ st\ +\ ss\ e^s}\ \frac{+}{+} \text{ - Full ♀}$$

$$\frac{F}{F}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ ss\ e^s}{+\ +\ +\ be\ +\ +}\ \frac{+}{+} \text{ - Full ♀}$$

$\frac{F}{-}\;\frac{+}{+}\;\frac{ru\ h\ st + ss\ e^{s}}{+ + + be + +}\;\frac{+}{+}$ - Full ♂

$\frac{F}{-}\;\frac{+}{+}\;\frac{ru\ h\ st + ss\ e^{s}}{ru\ h\ st + ss\ e^{s}}\;\frac{+}{+}$ - Full ♂

$\frac{F}{F}\;\frac{+}{+}\;\frac{+ + + be + +}{ru\ h\ st + ss\ e^{s}}\;\frac{+}{+}$ - Full ♀

$\frac{F}{F}\;\frac{+}{+}\;\frac{+ + + be + +}{+ + + be + +}\;\frac{+}{+}$ - Full ♀

$\frac{F}{-}\;\frac{+}{+}\;\frac{+ + + be + +}{+ + + be + +}\;\frac{+}{+}$ - Full ♂

$\frac{F}{-}\;\frac{+}{+}\;\frac{+ + + be + +}{ru\ h\ st + ss\ e^{s}}\;\frac{+}{+}$ - Full ♂

$\frac{B}{F}\;\frac{+}{+}\;\frac{+ + + be + +}{ru\ h\ st + ss\ e^{s}}\;\frac{+}{+}$ - Het. ♀

$\frac{B}{F}\;\frac{+}{+}\;\frac{+ + + be + +}{+ + + be + +}\;\frac{+}{+}$ - Het. ♀

$\frac{B}{-}\;\frac{+}{+}\;\frac{+ + + be + +}{+ + + be + +}\;\frac{+}{+}$ - bean ♂ (with wild characters)

$\frac{B}{-}\;\frac{+}{+}\;\frac{+ + + be + +}{ru\ h\ st + ss\ e^{s}}\;\frac{+}{+}$ - Bar ♂

$\frac{B}{F}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ ss\ e^{s}}{ru\ h\ st\ +\ ss\ e^{s}}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{F}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ ss\ e^{s}}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{\text{—}}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ ss\ e^{s}}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$ - Bar ♂

$\frac{B}{\text{—}}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ ss\ e^{s}}{ru\ h\ st\ +\ ss\ e^{s}}\ \frac{+}{+}$ - Bar ♂

Fig. 13 - Inheritance of bean, assuming bean is in Chromosome III.

Heterozygous ♀ × Full ♂ (F_1's of previous cross).

(It is assumed break in chromosomes takes place between "st" + "+").

$$\frac{F}{B}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ +\ +}{+\ +\ +\ be\ ss\ e^s}\ \frac{+}{+}$$

×

$$\frac{F}{-}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ ss\ e^s}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$$

$$\Big\|$$

$$\frac{F}{F}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ +\ +}{ru\ h\ st\ +\ ss\ e^s}\ \frac{+}{+}$$ - Full ♀

$$\frac{F}{F}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ +\ +}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$$ - Full ♀

$$\frac{F}{-}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ +\ +}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$$ - Full ♂

$$\frac{F}{-}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ +\ +}{ru\ h\ st\ +\ ss\ e^s}\ \frac{+}{+}$$ - Full ♂

$$\frac{B}{F}\ \frac{+}{+}\ \frac{+\ +\ +\ be\ ss\ e^s}{ru\ h\ st\ +\ ss\ e^s}\ \frac{+}{+}$$ - Het. ♀

$$\frac{B}{F}\ \frac{+}{+}\ \frac{+\ +\ +\ be\ ss\ e^s}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$$ - Het. ♀

$\frac{B}{\text{—}}\ \frac{+}{+}\ \frac{+\ +\ +\ be\ ss\ e^s}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$ - bean ♂ (with wild characters)

$\frac{B}{\text{—}}\ \frac{+}{+}\ \frac{+\ +\ +\ be\ ss\ e^s}{ru\ h\ st\ +\ ss\ e^s}\ \frac{+}{+}$ - Bar ♂

$\frac{F}{F}\ \frac{+}{+}\ \frac{+\ +\ +\ be\ ss\ e^s}{ru\ h\ st\ +\ ss\ e^s}\ \frac{+}{+}$ - Full ♀

$\frac{F}{F}\ \frac{+}{+}\ \frac{+\ +\ +\ be\ ss\ e^s}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$ - Full ♀

$\frac{F}{\text{—}}\ \frac{+}{+}\ \frac{+\ +\ +\ be\ ss\ e^s}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$ - Full ♂

$\frac{F}{\text{—}}\ \frac{+}{+}\ \frac{+\ +\ +\ be\ ss\ e^s}{ru\ h\ st\ +\ ss\ e^s}\ \frac{+}{+}$ - Full ♂

$\frac{B}{F}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ +\ +}{ru\ h\ st\ +\ ss\ e^s}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{F}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ +\ +}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$ - Het. ♀

$\frac{B}{\text{—}}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ +\ +}{+\ +\ +\ be\ +\ +}\ \frac{+}{+}$ - Bar ♂

$\frac{B}{\text{—}}\ \frac{+}{+}\ \frac{ru\ h\ st\ +\ +\ +}{ru\ h\ st\ +\ ss\ e^s}\ \frac{+}{+}$ - Bar ♂

Fig 14 - Inheritance of bean, when crossing-over takes place in ♀.

Since the bean gene modifier has been eliminated from chromosomes I, II, and III it follows that it must be a member of the fourth linkage group, and crosses are now in progress to determine whether this is so.

NON-APPEARANCE OF BEAN-EYED FEMALE:

So far the question of the non-appearance of the bean-eyed female has been ignored. Examination of the records will show that no female corresponding to the bean-eyed male has been obtained. At first it was thought that the bean gene modifier might be linked to a lethal factor and that possibly close imbreeding for succdessive generations might break down this linkage. However, five successive generations were bred and innumerable flies examined but no bean-eyed female appeared. Again, if the non-appearance of the bean-eyed female were due to this fact the normal 1:1 sex-ratio should be disturbed and this was not the case.

An examination of the chromosomal formulae of the F_2 classes of the II-ple crosses may help to solve the problem. From the results of the YY female x bean male cross the formula of the bean-eyed male is assumed to be:

$$\frac{B}{\quad} \; \frac{+}{+} \; \frac{+}{+} \; \frac{be}{be}$$

where B = Bar and be = bean. The formulae for the full and bar-eyed flies are known to be:

$$\frac{F}{F}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{+}\ \text{- Full ♀} \qquad \frac{B}{B}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{+}\ \text{- Bar ♀}$$

$$\frac{F}{\ }\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{+}\ \text{- Full ♂} \qquad \frac{B}{\ }\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{+}\ \text{- Bar ♂}$$

A study of Fig.10 will show that the gene for the bean modifier does not occur in any of the F_1 offspring in the homozygous condition and the eyes of the offspring indicate that the bean gene

1. is recessive to its normal allelomorph,

or 2. has no effect in single dose.

A study of the F_2 generation (Fig.10,p.27) shows that here the bean gene occurs in the homozygous condition in twelve cases, an analysis of which follows:

1. Nos. 1, 2, 5 and 6 carry the gene for Full eye in both their X-chromosomes but in spite of the fact that they carry the bean modifier in the homozygous condition the full-eyed females did not seem to differ from the normal full-eyed flies.
2. Nos. 3, 4, 7 and 8 carry the gene for Full in their single X-chromosome but here again these

full-eyed individuals did not appear to differ from the wild type. However, facet counts were not made so that it cannot be stated definitely that no change in this respect was effected, but there was no nicking in the anterior margin of the eye.

3. Nos. 9, 10, 13 and 14 carry the gene for Full in one X-chromosome and the Bar gene in the other. There is always considerable variation in the shape of the eye of such hybrid females and it is therefore impossible to estimate the effect of the bean gene, if any, in these cases.
4. Nos. 11, 12, 15 and 16 represent males which carry the Bar gene in their single X-chromosome and these individuals are presumably the bean-eyed males obtained.

In view of this, then, it is not surprising that no bean-eyed females were obtained. Further light may, however, be thrown on the question by a re-examination of the results of the first crosses made, i.e. between the bar-eyed female and the bean-eyed male, as shown in fig. 15.

Bar♀ x bean ♂

$$P_1 \quad \frac{B}{B}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{+}$$

$$\times$$

$$\frac{B}{—}\ \frac{+}{+}\ \frac{+}{+}\ \frac{be}{be}$$

$$\|$$

$$F_1 \quad \frac{B}{B}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{be} \text{ - Bar ♀}$$

$$\times$$

$$\frac{B}{—}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{be} \text{ - Bar ♂}$$

$$\|$$

$$F_2 \quad \frac{B}{B}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{+} \text{ - Bar ♀}$$

$$\frac{B}{B}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{be} \text{ - Bar ♀}$$

$\frac{B}{-}\;\frac{+}{+}\;\frac{+}{+}\;\frac{+}{be}$ - Bar ♂

$\frac{B}{-}\;\frac{+}{+}\;\frac{+}{+}\;\frac{+}{+}$ - Bar ♂

$\frac{B}{B}\;\frac{+}{+}\;\frac{+}{+}\;\frac{be}{+}$ - Bar ♀

$\frac{B}{B}\;\frac{+}{+}\;\frac{+}{+}\;\frac{be}{be}$ - Bar ♀

$\frac{B}{-}\;\frac{+}{+}\;\frac{+}{+}\;\frac{be}{be}$ - bean ♂

$\frac{B}{-}\;\frac{+}{+}\;\frac{+}{+}\;\frac{be}{+}$ - Bar ♂

Fig 15 - Inheritance of bean, assuming bean is in Chromosome IV.

There is nothing unusual about the F_1 offspring but in the F_2 generation there are two individuals, one male and one female, which have the bean gene modifier in the homozygous condition. Nevertheless, no bean-eyed females were obtained from these crosses. A contrast of the chromosomal constitution of these two individuals is interesting. In the case of the female, the Bar gene is present in both X-chromosomes giving a ratio of 1 Bar gene to 1 bean gene. In the male, on the other hand, with only one Bar gene in its single X-chromosome, the ratio is 1 Bar gene to 2 bean genes. Since no bean-eyed females were obtained it is possible that this may be the explanation of their non-appearance. It may well be that the bean modifier cannot gain expression in the face of the two Bar genes of the female.

When the Bar-bean cross was originally made a ratio of 1 bean-eyed fly to 3 Bar-eyed flies was expected as it was assumed that bean was a sex-linked recessive. However, from Fig.15 it will be seen that the ratio of bean-eyed flies to bar-eyed flies should be 1:7 and a reference to the results of these crosses (see p.7) will show that this ratio is very closely approximated, which would seem to confirm the assumptions stated above.

RATIO OF BEAN-EYED FLIES TO REST OF POPULATION:

Another point that remains to be considered is the percentage of bean-eyed flies obtained in the various crosses. In the case of the II-ple crosses, Fig.10 shows that the ratio should be 1 bean-eyed male fly to 15 other flies and an examination of the results from the II-ple-bean crosses shows that in some of the crosses this ratio is almost obtained. (See p.25). A total of 7,679 flies were examined, of which 461 were bean-eyed males, which gives a ratio of 1:16. It is very probable that if more crosses had been made, and consequently more flies examined, the 1:15 ratio would have been obtained.

As has already been stated, the number of bean-eyed males obtained from the first III-ple x bean crosses was very small but later crosses gave a much higher proportion.

With regard to the YY-bean crosses, so far only the F_1 generation has been considered. However, when these F_1's are permitted to interbreed, a number of bean-eyed males are found among the F_2 generation and, as Fig.16 shows, the ratio of such flies to the other flies should be 1:7. Actually, in a cross of this type, a ratio of 1:8 was obtained, as shown below:

YY♀ x bean ♂

F_1 Generation:

Bar ♂	Full ♀
34	22

F_2 Generation:

Full ♀	Bar ♂	Bean ♂
247	220	58

$\underbrace{247 \quad 220}$

467 : 58

8 : 1

YY ♀ × bean ♂

$$P_1 \quad \frac{(XX)\ F}{(Y)\ —}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{+}$$

×

$$\frac{(X)\ B}{(Y)\ —}\ \frac{+}{+}\ \frac{+}{+}\ \frac{be}{be}$$

‖

$$F_1 \quad \frac{(XX)\ F}{(X)\ B}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{be}$$ – 3-X ♀ - does not develop.

$$\frac{(XX)\ F}{(Y)\ —}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{be}$$ – Full ♀

×

$$\frac{(X)\ B}{(Y)\ —}\ \frac{+}{+}\ \frac{+}{+}\ \frac{be}{+}$$ – Bar ♂

$$\frac{(Y)\ —}{(Y)\ —}\ \frac{+}{+}\ \frac{+}{+}\ \frac{+}{be}$$ – 2-Y ♂ - does not develop.

‖

F_2

$\begin{array}{c}(XX)\\(X)\end{array}\frac{F}{B}\;\frac{+}{+}\;\frac{+}{+}\;\frac{+}{be}$ - 3-X ♀ - does not develop.

$\begin{array}{c}(XX)\\(X)\end{array}\frac{F}{B}\;\frac{+}{+}\;\frac{+}{+}\;\frac{+}{+}$ - 3-X ♀ - does not develop.

$\begin{array}{c}(XX)\\(Y)\end{array}\frac{F}{-}\;\frac{+}{+}\;\frac{+}{+}\;\frac{+}{+}$ - Full ♀

$\begin{array}{c}(XX)\\(Y)\end{array}\frac{F}{-}\;\frac{+}{+}\;\frac{+}{+}\;\frac{+}{be}$ - Full ♀

$\begin{array}{c}(XX)\\(X)\end{array}\frac{F}{B}\;\frac{+}{+}\;\frac{+}{+}\;\frac{be}{be}$ - does not develop.

$\begin{array}{c}(XX)\\(X)\end{array}\frac{F}{B}\;\frac{+}{+}\;\frac{+}{+}\;\frac{be}{+}$ - 3-X ♀ - does not develop.

$\begin{array}{c}(XX)\\(Y)\end{array}\frac{F}{-}\;\frac{+}{+}\;\frac{+}{+}\;\frac{be}{+}$ - Full ♀

$\begin{array}{c}(XX)\\(Y)\end{array}\frac{F}{-}\;\frac{+}{+}\;\frac{+}{+}\;\frac{be}{be}$ - Full ♀

$\begin{array}{c}(X)\\(Y)\end{array}\frac{B}{-}\;\frac{+}{+}\;\frac{+}{+}\;\frac{be}{be}$ - bean ♂

$$\begin{matrix}(X)\\(Y)\end{matrix}\ \frac{B}{}\quad\frac{+}{+}\quad\frac{+}{+}\quad\frac{+}{be}\qquad - \text{Bar } ♂$$

$$\begin{matrix}(Y)\\(Y)\end{matrix}\ \frac{}{}\quad\frac{+}{+}\quad\frac{+}{+}\quad\frac{be}{+}\qquad - \text{2·Y·}♂ \text{ - does not develop.}$$

$$\begin{matrix}(Y)\\(Y)\end{matrix}\ \frac{}{}\quad\frac{+}{+}\quad\frac{+}{+}\quad\frac{be}{be}\qquad - \text{2-Y }♂ \text{ - does not develop.}$$

$$\begin{matrix}(X)\\(Y)\end{matrix}\ \frac{B}{}\quad\frac{+}{+}\quad\frac{+}{+}\quad\frac{be}{+}\qquad - \text{Bar } ♂$$

$$\begin{matrix}(X)\\(Y)\end{matrix}\ \frac{B}{}\quad\frac{+}{+}\quad\frac{+}{+}\quad\frac{+}{+}\qquad - \text{Bar } ♂$$

$$\begin{matrix}(Y)\\(Y)\end{matrix}\ \frac{}{}\quad\frac{+}{+}\quad\frac{+}{+}\quad\frac{+}{+}\qquad - \text{2-Y }♂ \text{ - does not develop.}$$

$$\begin{matrix}(Y)\\(Y)\end{matrix}\ \frac{}{}\quad\frac{+}{+}\quad\frac{+}{+}\quad\frac{+}{be}\qquad - \text{2-Y }♂ \text{ - does not develop.}$$

Fig 16 - Inheritance of bean with Non-disjunction ♀.

THE BEAN GENE AS A MODIFIER OF THE FULL GENE:

If the bean gene acted as a modifier of the Full gene as well as of the Bar gene the proportion of bean-eyed males in all the crosses would have to be much higher and it is therefore very unlikely that this is so. However, a number of bean-eyed males were observed which had a very much wider eye than the ordinary bean-eyed fly and these were mated to non-disjunction females. The following results were obtained:

	Full	Bar
1. F_1 -	♀	♂
	35	32

	Full	Bar
2. F_2 -	♀	♂
	36	32

As the males were bar-eyed it indicated that the X-chromosome of the parent had carried the Bar and not the Full gene.

LOCUS OF BEAN GENE:

The question of the determination of the locus of a gene has already been discussed. As stated, the distance between the genes determines the amount of crossing-over. Therefore, if the locus of one of these genes is known the locus of the other gene can be calculated from the amount of crossing-over that has taken place, this being proportional to the distance between the two genes. Crossing-over, however, only occurs in the female and since no bean-eyed females can be obtained it is impossible to determine the locus of this modifying gene.

CONCLUSIONS:

1. Bean is not sex-linked.
2. Bean is a modifier of the Bar gene but only gains expression in the male because here there is only one Bar gene while in the female there are two Bar genes.
3. Bean does not act as a modifier of the Full gene.
4. This bean gene modifier has been eliminated from the first, second and third linkage groups. It must, therefore, be a member of the fourth linkage group.
5. The locus of the bean modifier cannot be determined because crossing-over occurs only in the female and there are no bean-eyed females.

BIBLIOGRAPHY

1. Morgan, T.H. 1926. The Theory of the Gene.

2. Morgan, T.H., Bridges, C.B., Sturtevant, A.H. 1925. The Genetics of Drosophila.

3. Bridges, C.B. and Morgan, T.H. 1919. The second-chromosome group of mutant characters.

4. Ibid 1923. The third-chromosome group of mutant characters of Drosophila melanogaster.

5. Zeleny, Charles. 1917. Germinal changes in the Bar-Eyed Race of Drosophila during the Course of Selection for Facet Number.

A SYSTEMATIC STUDY OF THE LEECHES OF WESTERN CANADA.

The Hirudinea or leeches are annelids which occur in both fresh and salt water as well as on land. However, by far the greater number, are inhabitants of ditches, pools, ponds, lakes and streams. Some of the fresh-water species are known to leave the water in search of earthworms and one, *Haemopis lateralis terrestris*, has been found several miles from water. Permanent terrestrial leeches are reported from various points in Asia, Australia and South America. In the Himalayas they are found in great abundance in the lower ranges and attain an altitiude of ~~one~~ eleven thousand feet above sea level. The marine species are for the most part permanent ecto-parasites.

In this paper only the fresh-water leeches will be discussed. Very few lakes are without a few species of leeches and they are most commonly found in association with various species of snails, insect larvae and other annelids. They are for the most part nocturnal and during the day are to be found beneath stones, among plants or in the mud. Their food consists of blood and other animal juices and they are therefore semi-parasitic. Some, howeverm are nearly permanent parasites. They attack fish, frogs, turtles, snails, and occasionally fresh-water mussels. Several species will attack man.

The leeches represented in the collection were taken at various points in Manitoba, Saskatchewan, Alberta and British Columbia. Collecting for any length of time was, however, only done at Jasper Park, Alta. At some of the points a stop of only an hour or so was,made and the collection is, therefore, only suggestive of what may be found. Further work is certain to reveal many more species. The present collection does, however, establish the occurrence of the common species from coast to coast of Canada. The more northern lakes still remain practically untouched and the northern limits of these species is consequently unknown.

Of the four families of North American fresh-water leeches only three have been found or reported for Western Canada, the family Ichthyobdellidae being as yet unrepresented. The following are the species found:

I. Family Glossiphonidae
    A. Genus Helobdella R. Blanchard 1896.
        1. _H. stagnalis_
    B. Genus Glossiphonia Johnston 1816.
        2. _G. complanata_
        3. _G. heteroclita_
    C. Genus Placobdella R. Blanchard 1893.
        4. _P. rugosa_
    D. Genus Theromyzon Philippi 1867.
        5. _T. occidentalis_

II. Family Hirudinidae

E. Genus Macrobdella Verrill 1872.

6. M. decora

F. Genus Haemopis Savigny 1820.

7. H. grandis

8. H. marmoratis

9. H. plumbeous

III. Family Erpobdellidae

G. Genus Erpobdella Blainville 1818.

10. E. punctata

H. Genus Nephelopsis Verrill 1872.

11. N. obscura

I. Genus Dina R. Blanchard 1892.

12. D. parva.

As many of the organs and parts are of systematic value, it may be of interest to discuss, briefly, the various systems, but first of all the question of annulation will be considered. The body is made up of a number of annuli but, unlike the condition in, for example, the earthworm, the external annulation does not correspond to the real metamerism. Each true somite is made up of a number of annuli but this number differs in the various types of leeches. The number of somites, however, is the same for all species, viz. thirty-four. The number of annuli in a somite varies in different parts of the body but what are known as "complete somites" are to be found in the middle of the body. In the Glossiphonidae the number varies from two to six but most of the species, i.e. those of the more common genera, are essentially triannulate. The complete somites of the Hirudinidae and ~~He~~Erpobdellidae have five annuli, although in some of the genera of the latter family, viz. Nephelopsis and Dina, annulus b6 is enlarged and subdivided. These differences are illustrated in figs.1, 2, 3 and 4.

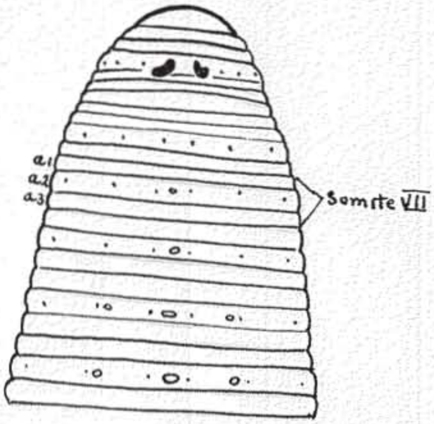


Fig. 1 - Annulation of a Triannulate Glossiphonid.

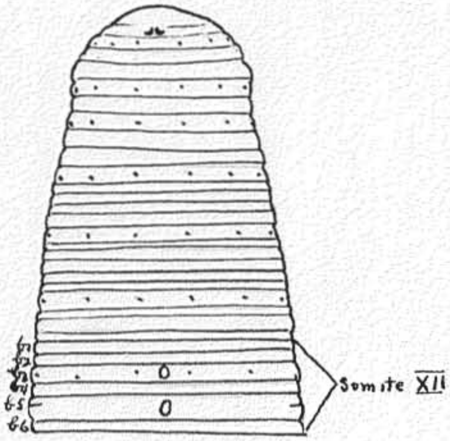


Fig. 2 - Annulation of Actinobdella inequiannulata - showing somite with 6 annuli.

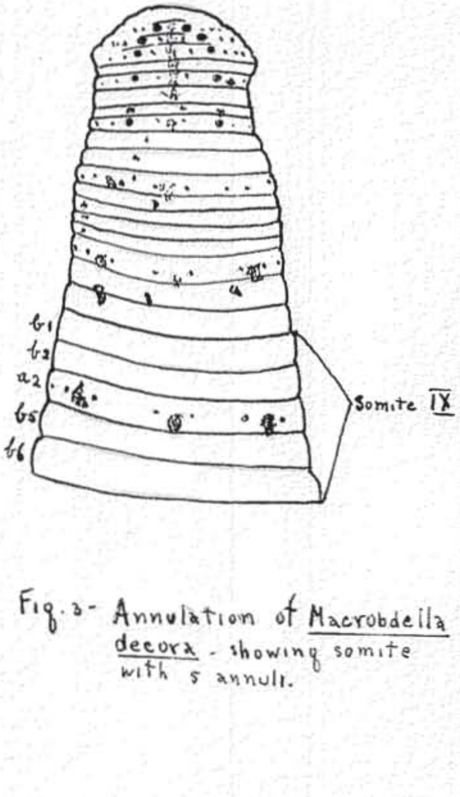


Fig. 3 - Annulation of Macrobdella decora - showing somite with 5 annuli.

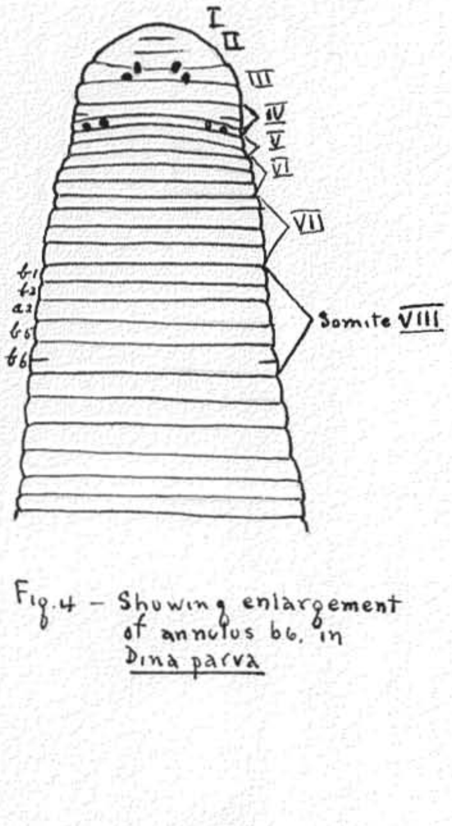


Fig. 4 - Showing enlargement of annulus b6, in Dina parva

The muscular system is well developed and consists of two layers of muscle fibres, the inner longitudinal, the outer circular. There are also dorso-ventral fibres passing vertically between the pouches of the crop and radial fibres extending from the wall of the alimentary canal to the body wall.

Respiration is almost entirely performed by the skin there being no special respiratory organs. In two marine genera, Branchellion and Ozobranchus, however, gills are present on certain somites. The capillaries are very close to the cuticle and the blood is thus brought into close relation with the surrounding water.

The circulatory system consists in general of seven longitudinal blood vessels, from which branches pass to the various organs, breaking up eventually into capillaries in the integument, the nephridia, etc.

The digestive system consists of the alimentary canal, differentiated into a number of parts, and the salivary glands. The mouth, which is situated ventrally, is, in the Rhynchobdellae, a mere pore in the oral sucker, while in the Gnathobdellae it is large and occupies practically the entire cavity of the sucker. The sucker itself is reduced to a mere lip. (See figs.5 and 6).

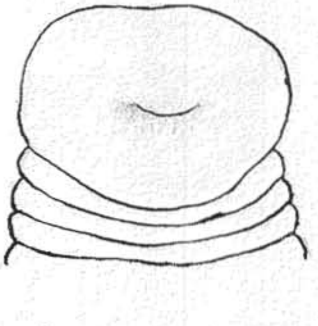


Fig.5- Mouth and Oral Sucker of Placobdella sp.

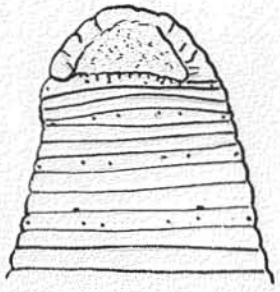


Fig.6 - Mouth and Oral sucker of Haemopis plumbeous

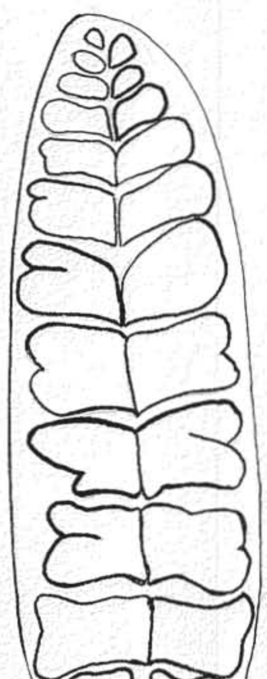


Fig.7 - Surface view of median jaw of H. marmoratis

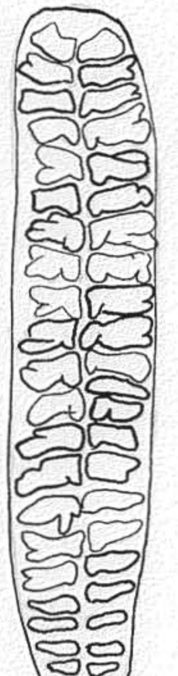


Fig.8 - Surface view of median jaw of Haemopis lateralis.

In some of the members of the latter sub-order the mouth is provided with three "jaws". The free edge of each jaw is curved and covered with chitin which, in Macrobdella decora, is finely notched there being about sixty-five teeth. In the genus Haemopis the teeth are much coarser, H. marmoratis having twelve to sixteen pairs and H. lateralis twenty to twenty-five, as shown in figs. 7 and 8.

The mouth leads into a muscular pharynx. Muscles pass from this region to the body wall and by their contraction increase the cavity and suck in the blood from the wounds made by the jaws. In the Rhynchobdellae the pharynx can be protruded and is termed a proboscis. Opening into the pharynx are a large number of unicellular glands, the salivary glands, which secrete a fluid which prevents the coagulation of the blood taken from the host.

The stomach or crop follows the pharynx and forms the largest portion of the alimentary canal. It varies in the different groups in accordance with the habits of the leech. In those species which subsist on the blood of other animals the crop has a number of diverticula known as the gastric caeca. In the Glossiphonidae the number varies from six to nine pairs. In the genera Helobdella and Glossiphonia the caeca are unbranched but in the genera Placobdella and *Theromyzon* they are often much branched. In the Hirudinidae, the family to which the true bloodsuckers belong, the caeca vary considerably. *Macrobdella decora* has about eighteen pairs, the last of which is very long. In *Haemopis marmoratis* and *H. lateralis*, along the entire length of the stomach are numerous small pouches with a very large pair at the posterior end. In *H. plumbeous* and *H. grandis*, there is hardly any indication of diverticula and even the posterior pair is very short. These differences are illustrated in figures 9, 10 and 11. The stomach of the Erpobdellidae, including the genera *Erpobdella*, *Nephelopsis* and *Dina*, is quite straight and simple, being without any diverticula, as shown in fig. 11.

The intestine, in the true bloodsuckers, may also have diverticula, which are termed the intestinal caeca. (See fig.9). Otherwise, the intestine is a simple tube, the posterior portion of which is sometimes differentiated when it is called the rectum. Either intestine or rectum open to the exterior through a small pore, the anus, situated mid-dorsally on or near the boundary between the body and caudal sucker.

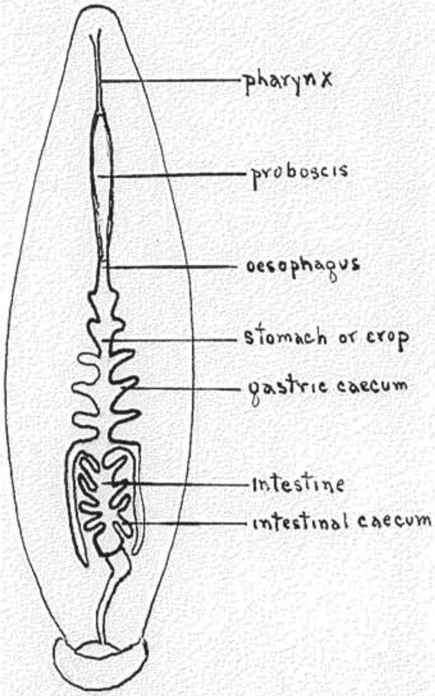


Fig. 9 - Alimentary canal of Glossiphonia sp.

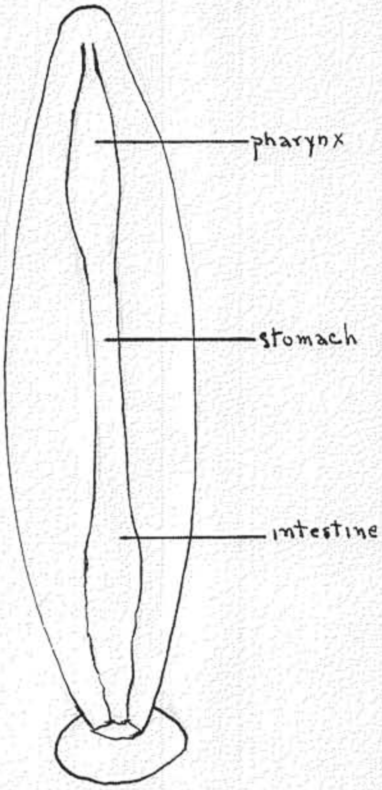


Fig. 11 - Alimentary canal of Nephelopsis obscura

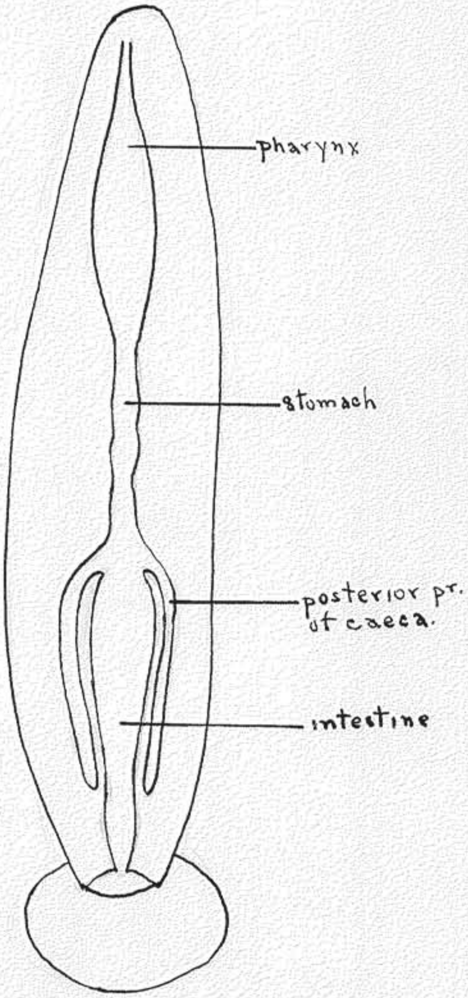


Fig. 10 - Alimentary canal of Haemopis marmoratis.

The excretory system consists of a pair of nephridia in each somite except those at the anterior and posterior ends. The nephridia open to the outside through a small pore, the nephridiopore, on the ventral side of, the body.

Leeches are hermaphroditic, the male and female reproductive organs being in the same individual. Cross-fertilization, however, takes place. The organs of the male consist of a varying number of pairs (5 to 11) of testes, each series being connected by a vas deferens. The essential female organs consist of a pair of ovaries, from each of which a short oviduct passes out. The genital apertures, of which the male pore is situated more anteriorly, are separated by from one to five annuli. In the various species of Helobdella and Placobdella there are two annuli intervening between these pores, as shown in fig.12; in Glossiphonia heteroclita there is only one annulus between these apertures but in G. complanata there are two. In the Hirudinidae the pores are separated by five annuli and in the Erpobdellidae the number varies from two to three and one-half. It is two in Nephelopsis obscura and Erpobdella punctata but three and one-half in Dina parva. (See figs.12, 13 and 14).

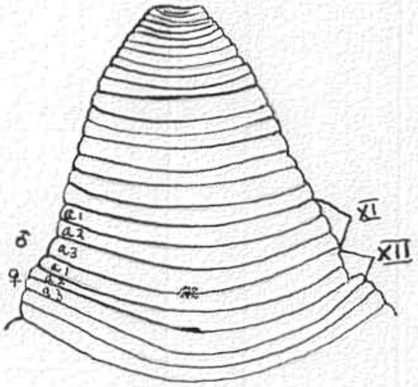


Fig. 12 - Genital apertures of <u>Placobdella sp.</u>

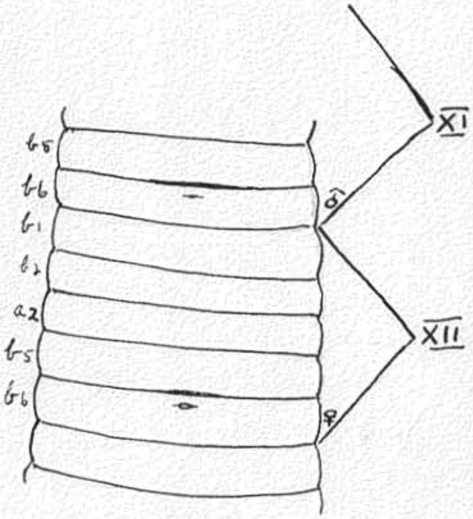


Fig. 13 - Genital apertures of <u>Haemopis marmoratis</u>

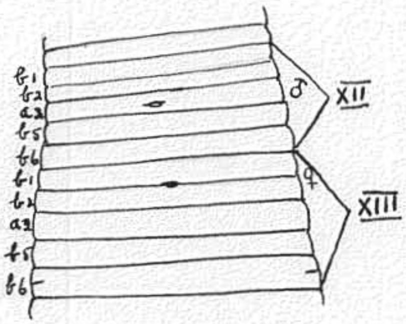


Fig. 14 - Genital apertures of <u>Dina parva.</u>

In the species of Haemopis there is considerable difference in the relations of the various organs comprising the reproductive system, as shown in figures 15, 16 and 17.

The form of the atrium of the groups of the family Erpobdellidae is of generic value, as shown in figs. 18-21.

The Glossiphonidae carry their young in membranous capsules attached to the venter. The eggs of the Ichthyobdellidae, the Hirudinidae, and the Erpobdellidae are laid in chitinoid cocoons in which the young develop.

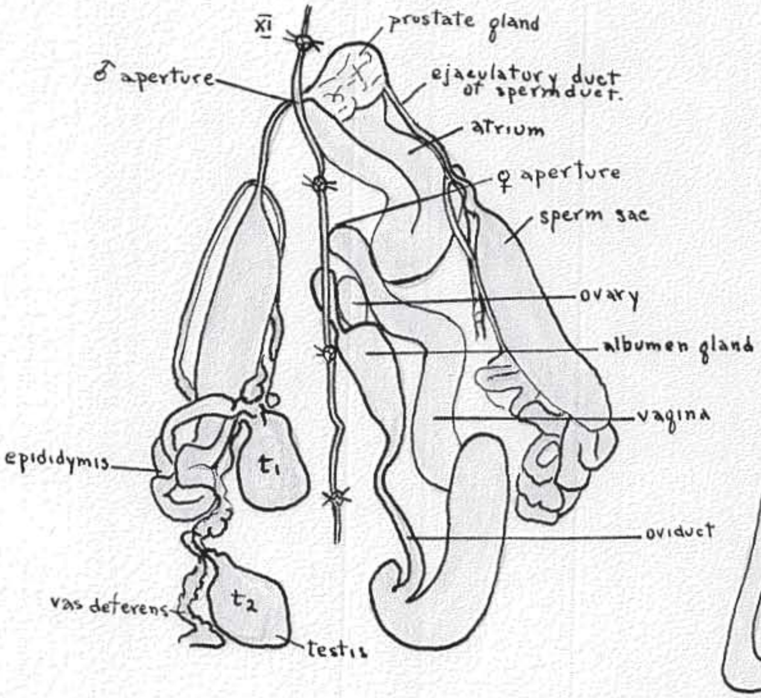


Fig. 15 - Reproductive organs of Haemopis grandis.

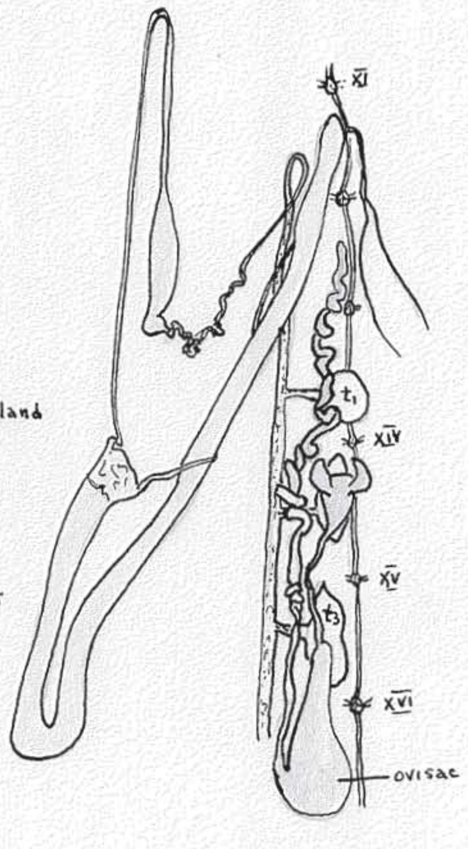


Fig. 16 - Reproductive organs of H. marmoratis.

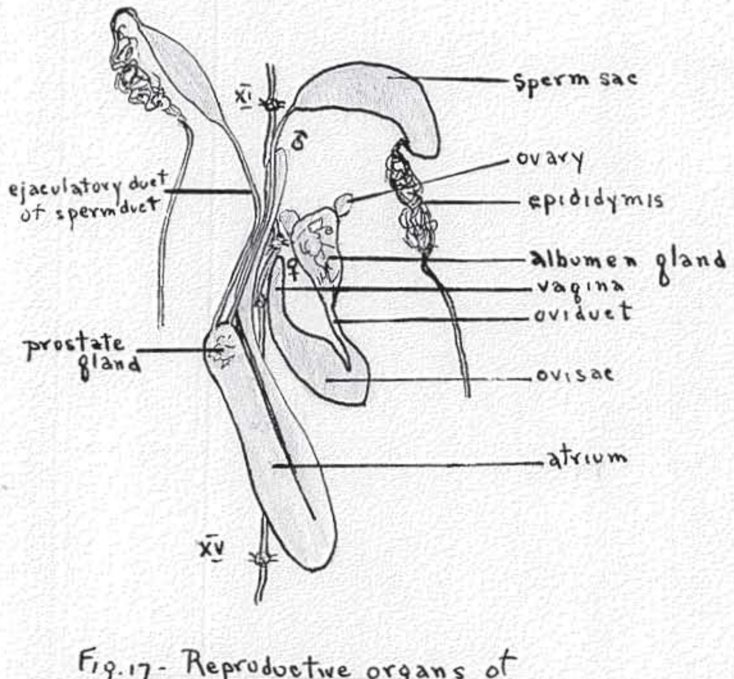


Fig. 17 - Reproductive organs of H. plumbeous

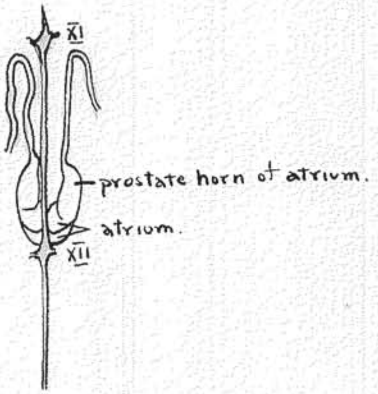


Fig. 18- Atrium of *Erpobdella punctata*.

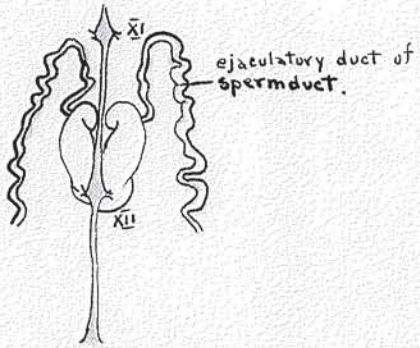


Fig. 19 - Atrium of *Nephelopsis obscura*.

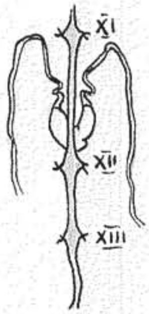


Fig. 20- Atrium of *Dina parva*.

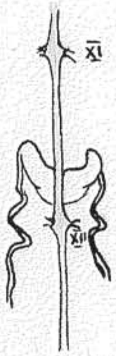


Fig. 21 - Atrium of *Dina fervida*.

The nervous system consists of a series of paired ganglia connected by double nerves. There is a pair of ganglia to each somite and the annulus in which the ganglia occur is termed the neural annulus. Three pairs of nerves pass from each pair of ganglia to the other annuli of the somite.

The principal sense organs are the eyes, the number of which is often of specific value. In the Rhynchobdellae the number varies from one to four pairs, as shown in figures 22-25; in the Gnathobdellae the number varies from three to five pairs, being three or four pairs in the Erpobdellidae and five pairs in the Hirudinidae, as shown in figures 26-28. In addition to the eyes, which are particularly sensitive to changes in the intensity of light, there are sensillae which are usually found on the neural annuli (see figs.1-3) and papillae which are either metameric or non-metameric, round or conical. The body as a whole seems to be sensitive to wave movements and light stimuli.

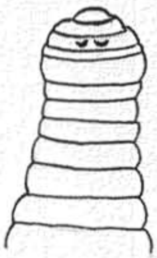


Fig. 22 - Eyes of Helobdella stagnalis.

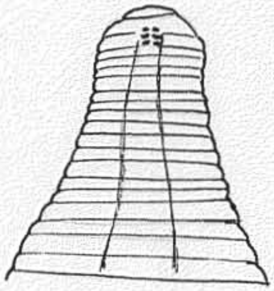


Fig. 23 - Eyes of Glossiphonia complanata.

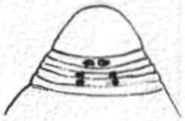


Fig. 24 - Eyes of G. heteroclita

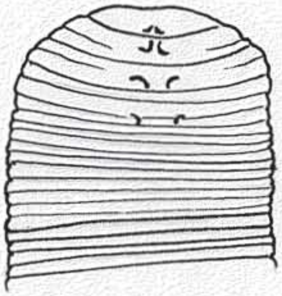


Fig. 25 - Eyes of Theromyzon occidentalis.

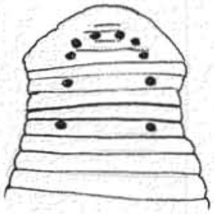


Fig. 26 - Eyes of Macrobdella decora

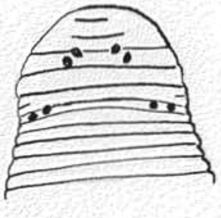


Fig. 27 - Eyes of Dina parva.


Fig. 28 - Eyes of Erpobdella punctata.

A description, with full synonymy, of the leeches which have been collected follows:

1. Helobdella stagnalis (Linn.)1758.

Hirudo stagnalis Linnaeus 1758.
Hirudo bioculata Bergmann 1757.
Glossiphonia stagnalis (Linn.) Johnston 1816.
Clepsine modesta Verrill 1872.
Helobdella stagnalis Blanchard 1896.

This leech, one of the most commonly found, is a quite small, inconspicuous animal, Usually it is only slightly pigmented and blends into the stones to which it is almost invariably attached. Its distinguishing feature is the presence, on the dorsum of the twelfth annulus, of a small, round, light brown, chitinoid plate which marks the position of the nuchal gland.

The anterior end of this leech is definitely narrower, the body widening gradually as it passes backwards. The ground colour in all specimens is creamy and, while in some individuals there is scarcely any pigmentation, in the majority of cases the posterior two-thirds of the body is covered with either fine, dark stippling or with light green blotches, giving a green tinge to the animal.

The single pair of eyes are very prominent, being heavily pigmented. The genital pores are separated by a single annulus. No papillae have been observed on any of the specimens. H. stagnalis does not swim but is an active creeper. When disturned it rolls into a ball and sinks to the bottom. Its food consists of small annelids, insect larvae, snails and small bivalves. Specimens have also been found on the dead bodies of crustaceans, frogs and fish, and when gorged with blood the six pairs of gastric caeca can be seen very clearly.

This leech has been found at the following points:

Logan West, Man.
Pelican Lake, Man.
Falcon River (Waugh, Man.)
Wascana Lake (Regina, Sask.)
Chestermere Lake (Calgary, Alta.
Vermillion Lakes } Banff, Alta.
Johnston Lake }
Shewatlans Lake (Prince Rupert, B.C.)
Diver Lake, (Wellington, Vancouver Is.)
Sproat Lake (Vancouver Island)
Tuktukum Creek (Departure Bay, Vancouver Is.)
Little Qualicum River (Vancouver Is.)
Jasper Park, Alta. - Lakes Caledonia, Marjorie, Pyramid, Big Trefoil, a lake north of Geikie, and Pyramid Cr.

2. *Glossiphonia complanata* (Linn.) 1758.

Hirudo complanata Linnaeus 1758.
Clepsine elegans Verrill 1874.

In the adult stage *G. complanata* is very easily recognisable because of the two dark lines that stand out clearly against the usually light background of the dorsal surface of the body. This species is larger and more robust than *H. stagnalis*. The majority of specimens are a light green in colour, although light brown and brownish green individuals do occur. Closer examination shows that the body is stippled with fine dark spots. The longitudinal lines referred to above are dark brown and are interrupted by the white papillae which occur on the neural annuli. These lines usually commence about the level of the second pair of eyes and diverge as they pass backwards. Often they become quite faint towards the posterior end of the body. The ventral surface is usually lighter and the longitudinal lines seem to diverge more. The three pairs of eyes form two nearly parallel lines although in some specimens the first pair are very close together. The genital apertures are separated by two annuli. There are at least four series of papillae, often six or seven.

Young individuals do not present as characteristic an appearance. The green pigment is not present and the longitudinal lines are not as prominent. The body of these young specimens is, however, very transparent and the seven pairs of caeca show very clearly when filled with blood.

Specimens of G. complanata have been taken at the following points:

Logan West, Man.

Falcon River (Waugh, Man.)

Shoal Lake (Waugh, Man.)

Victoria Beach, Lake Winnipeg.
Lake Winnipegosis, (Cormorant Is.)
Pelican Lake (Ninette, Man.)

Lake of the Woods (Minaki, Ont.)

Wascana Lake (Regina, Sask.)

Chestermere Lake (Calgary, Alta.)

Johnston Lake (Banff, Alta.)

Jasper Park - Lakes Beauvert, Big Trefoil, Marjorie, Maligne, Iris, a lake north of Geikie, and in Caledonia and Pyramid Creeks.

Little Qualicum River (Vancouver Island).

3. Glossiphonia heteroclita (Linn.)

Hirudo heteroclita Linnaeus (1761)

Hirudo hyalina O. F. Müller 1774.

Clepsine hyalina Moquin-Tandon 1826.

Like H. stagnalis this is a very inconspicuous animal for it is not heavily pigmented. The ground colour is white with yellowish spots. The body is translucent and when the gastric caeca are full of blood the animal appears as a bright red spot. Of the six pairs of caeca the first five are simple but the sixth pair which are much longer have three side branches.

The distinguishing feature of this species is the eyes, of which there are three pairs. These are arranged in three groups of two and form a triangular figure. The genital apertures are separated by one annulus.

G. heteroclita would seem to be much less common in the lakes of Western Canada than the two Glossiphonids just described for it was taken last spring for the first time in Falcon River and Shoal Lake, not having been found the previous summer at Jasper Park where the collecting extended over several months. It has not been reported from any other points in Western Canada.

4. Theromyzon occidentalis (Philippi) 1867.
Clepsine occidentalis Verrill 1874.
Hemiclepsis occidentalis Verrill -
Protoclepsis occidentalis Livanow 1902.

The colour of this leech varies from quite a bright green to very dark with scattered yellow or orange spots. Most specimens have four papillae on the neural annuli, except those at the anterior end, in addition to those occurring indiscriminately over the body. The specimens from Maligne Lake differed from the others in being of a uniform dull green hue and being without papillae.

The body of this species is very soft and translucent. The four pairs of eyes, serially arranged, are the distinguishing feature. The genital apertures are separated by two annuli.

This species, while it has been found more often than G. heteroclita, cannot be said to be common and very rarely were more than one or two specimens taken at a time. It was found at the following points:

Falcon River (Waugh, Man.)
Pelican Lake (Ninette, Man.)
Lake of the Woods (Minaki, Ont.)
Skookum Lake (Wawanesa, Man.)
Chestermere Lake (Calgary, Alta.)
Jasper Park, Alta. - Lakes Marjorie, Little Trefoil,
Talbot and Maligne.
Diver Lake,(Wellington, Vancouver Island).

5. Placobdella rugosa (Verrill) 1874.
Clepsine ornata var. rugosa Verrill 1874.
Placobdella rugosa Moore 1901.

This species is one of the largest of the Glossiphonids. The rough appearance of the body is a very striking feature and is due to the large number of cutaneous papillae that cover it. These papillae are for the most part large and conical. There are five series which occur on the neural annuli, in addition to many smaller ones the positions of which are variable.

In colour P. rugosa is a more or less uniform dull brown or green. Very young specimens are a light green. There is a black, median dorsal stripe which is interrupted by the presence of the papillae.

The eyes are fairly small and contiguous. Both the oral and caudal suckers are well developed. The genital pores are separated by two annuli.

Owing to its dull colouring and the fact that it can assume a very flattened position this leech might be overlooked easily as it blends in well with the stones to which it is usually found attached.

P. rugosa has been collected at the following points:

Falcon River (Waugh, Man.)
Pelican Lake, Ninette, Man.)
Lake Winnipeg (Victoria Beach, Man.)
Skookum Lake (Wawanesa, Man.)
Near Beaver Lake, Alta.
Johnston Lake,(Banff, Alta.)
Pyramid Lake (Jasper Park, Alta.)
Shewatlans Lake (Prince Rupert, B.C.)

6. Macrobdella decora (Say).

Hirudo decora Say 1824.
Hirudo decora Leidy 1868.
Macrobdella decora Verrill 1872.

M. decora, known as the American medicinal leech, is readily recognisable because of the presence of orange or red spots along the median dorsal line. These, as well as the marginal black spots, are metamerically arranged. Dorsally, the ground colour varies from a light sage green to a deep olive green, while some specimens may be so dark as to seem black. The ventral surface is a rich orange, either plain or spotted with black.

M. decora has the five pairs of eyes characteristic of all Hirudinidae but here they are larger than in any of the species of Haemopis. Cutaneous papillare are absent and the surface of the body is therefore quite smooth.

Unlike many species of Haemopis, M. decora never voluntarily leaves the water, although it may live for weeks buried in the mud left by the drying up of ponds or pools. It lives on the blood of fish, frogs and turtles and small aquatic annelids and insect larvae have been found in the stomach. Frogs' eggs are also a favourite food. In addition, it will attack humans and cattle.

This species is found as a rule in standing water and in small ponds and lakes. The eggs are laid in cocoons which are usually deposited in the mud by the side of the pond.

This leech has been collected at the following points:

White Shell Lake, Man.
Shoal Lake, Man.
Lake of the Woods, Ont.
Lake Winnipeg, Man.
Qu'Appelle Valley, Sask.

7. Haemopis marmoratis (Say).

Hirudo marmorata Say 1824.
Aulastomum lacustre Leidy 1868.
Haemopis marmoratis Moore 1901.

Haemopis marmoratis or the horse leech, as it is commonly called, rarely exceeds six inches in length. Owing to the great development of botyroidal tissue the body is very soft and limp. The anterior sucker is relatively large and the lip broad but the posterior sucker is rather small and circular. The jaws are low and rounded and each bears from twelve to sixteen pairs of coarse teeth.

The colour of this leech is somewhat variable. The dorsal surface ranges from a very dark green to olive green but the black blotches may be so numerous as to allow the green ground to be seen only here and there. On the other hand, they may be absent altogether, but this latter condition is rare. The ventral surface is more or less similar but the blotches are grey in most examples rather than black thus producing a lighter effect.

Specimens of H. marmoratis were found on the under side of stones as well as in the soft clay forming the bank of Big Trefoil Lake. It was taken at the following points:

Lake Winnipeg (Victoria Beach)
Near Beaver Lake, Alta.
Vermillion Lakes, Banff, Alta.
Jasper Park, Alta.- Lakes Marjorie, Little Trefoil,
  Big Trefoil, Beauvert, Hibernia, Caledonia, and
  a lake north of Geikie.
Sproat Lake, Vancouver Island.
Falcon River, (Waugh, Man.)

8. Haemopis plumbeous Moore.

Haemopis plumbeous Moore 1912.

This species was first described by Moore in 1912. It resembles H. lateralis, a species which has as yet not been recorded for Canada, quite closely in colour but is more nearly allied to H. grandis in both internal and external structure. _H. plumbeous_ is represented in the present collection by a number of preserved specimens collected at Shoal Lake, Man., a number of years ago. The ground colour of the dorsal surface was probably a dull olive green but is almost entirely covered over by black pigment. The ground colour of the ventral surface appears darker but is much less blotched. This species is described by Moore as having a broad, dull but conspicuous rufous or orange band along the entire lateral margins. In the preserved specimens it is difficult to state whether this band is present although in some specimens there seems to be some faint indication of its presence.

The mouth is large even in these preserved specimens. In some of the individuals the penis is protruded and is thick and coiled.

9. _Haemopis grandis_ (Verrill)

Semiscolex grandis Verrill 1874.

Two types of this species are represented in this collection one of which, found in the lakes of Jasper Park, differs considerably in general appearance from the second variety, as well as from the other species of _Haemopis_. It is a small leech as compared with other members of this genus, the largest individual obtained being barely 8 cm. in length. In colour it varies from olive to dark green, plain or with a few dark spots. The ventral surface is a slaty grey and always plain. A yellowish green band which tapers off anteriorly, is present on the lateral margins.

The second type, in addition to being a much larger animal, differs in colouration, from the type just described. The ground colour of the dorsal surface is a dull green but owing to the widespread distribution of black pigment it shows only in spots. The ventral surface is similar but very much purer, there being only isolated black spots.

The distinguishing feature of this species of Haemopis is found in the reproductive organs - the proportion of the several regions of the ducts being quite different from all the other species of Haemopis. The penis when protruded is thin and straight.

This leech has been collected at the following points:

Shoal Lake (Waugh, Man.)
Pilot Mound, Man.
Lake of the Woods, Ont.
Cross Lake, Man.
Jasper Park, Alta.- Lakes Beauvert, Patricia,
Hibernia and Caledonia.

10. *Erpobdella punctata* (Leidy)

Nephelis punctata Leidy 1870.

Nephelis lateralis Bristol 1898.

Erpobdella punctata Moore 1901.

The two or four longitudinal bands on the dorsal surface of the body make recognition of this leech very simple. The three pairs of eyes are another characteristic feature. *E. punctata* may attain a length of five inches but the majority of specimens collected are smaller than this.

The colour ranges from olive green to light brown or chocolate. The black spots, as already stated, are arranged in longitudinal lines.

During the day this leech can be found attached to the under side of stones but it is a very active swimmer. It feeds chiefly on aquatic insects and their larvae and aquatic worms but also consumes the young of its own species.

It was not found to any extent in the lakes of Jasper Park but elsewhere it has been taken in large numbers, viz.:

Shoal Lake, Waugh, Man.
Falcon River, Waugh, Man.
Pelican Lake (Ninette, Man.)
Lake Winnipeg (Victoria Beach, Man.)
Wascana Lake (Regina, Sask.)

Chestermere Lake (Calgary, Alta.)

Vermillion Lakes (Banff, Alta.)

Jasper Park - Lakes Annette, Edith, Mildred, Pyramid, Patricia, Marjorie, Caledonia, Dorothy, a lake north of Geikie, and in Pyramid Creek.

Tuktukum Creek (Departure Bay, Vancouver Is.)

Cameron Lake (Vancouver Island.)

Little Qualicum River (Vancouver Is.)

Diver Lake (Wellington, Vandouver Is.)

Horn " " "

Sproat Lake, Vancouver Is.

11. *Nephelopsis obscura* Verrill

Nephelopsis obscura Verrill 1872.

There is considerable variation in the colour of this species but four main types can be recognised:

1. Dorsal surface - ground olive green with black ramifications. Ventral surface - slaty grey, plain or with dark blotches.
2. Dorsal surface - ground, pea green with lighter coloured blotches. Ventral surface - lighter, plain.
3. Dorsal surface - brownish grey, plain. Ventral surface - light grey, plain.
4. Dorsal surface - very dark grey with occasional light grey blotches. Ventral surface - light grey, plain.

Of these four varieties the first is by far the most common. N. obscura attains a large size and like N. punctata is a very graceful swimmer. The body is very firm and flattened posteriorly.

The egg cases are found in great numbers attached to stones and aquatic plants. The young, removed from the cases, are quite colourless and the four pairs of eyes show very clearly.

Although so common in the lakes of Manitoba and Alberta, no specimens of this leech were found in the lakes of Vancouver Island that were examined. It was collected at the following points:

Logan West, Man.
Falcon River and Shoal Lake (Waugh, Man.)
Pelican Lake (Ninette, Man.)
Skookum Lake (Wawanesa, Man.)
Johnston Lake and Vermillion Lakes (Banff, Alta.)
Near Beaver Lake, Alta.
Jasper Park, Alta.- Lakes Annette, Edith, Mildred, Beauvert, Big Trefoil, Pyramid, Patricia, Hibernia, Marjorie, Dorothy, a lake north of Geikie, and Caledonia Creek.

12. Dina parva Moore 1912.

This leech is of a small size, the largest specimens reaching only a length of 20 mm. In colour it is a plain olive green. The four pairs of eyes are easily made out even in the preserved material and in this respect it differs from *N. obscura*.

The distinguishing feature of *D. parva* is the fact that the genital pores are separated by three and one-half annuli - in both *N. obscura* and *E. punctata* the distance between these pores is only two annuli.

D. parva is represented in the collection by specimens from only two points - Jasper Park (Maligne Lake) and Lake Winnipegosis, Man. (Cormorant Is.)

Key to Species Descfibed

A. Mouth a small pore in oral sucker from which a muscular proboscis may be protruded; no jaws; size small.
Suborder Rhynchobdellae.

a. Body not divided into two regions; eyes near median line; stomach with well developed lateral caeca.
Family Glossiphonidae.

1. Eyes, 4 prs. *Theromyzon occidentalis*

2. Eyes, 3 prs. arranged in two nearly parallel rows.
Glossiphonia complanata.

3. Eyes, 3 prs. arranged in three groups of two forming a triangular figure.
Glossiphonia heteroclita

4. Eyes, 1 pr. well separated. Brown chitinoid plate at annulus 12.
Helobdella stagnalis.

5. Eyes, 1 pr. close together.
Placobdella rugosa

B. Mouth large occupying entire cavity of sucker; pharynx not forming a proboscis; jaws often present.
Suborder Gnathobdellae.

b. Eyes typically 5 prs. arranged in a regular submarginal arch; testes strictly paired, 9 or 10 prs.; stomach with at least 1 pr. of gastric caeca; size generally large.
Family Hirudinidae.

b'. Jaws prominent; teeth num~~b~~erous, in one series; caeca along entire length of stomach.
Genus Macrobdella.

6. About 65 teeth on each jaw; copulatory glands present; dorsum marked with median red and marginal black spots, both metameric.
Macrobdella decora

b''. Jaws rather small and retractile into pits or absent; teeth when present coarse and all in double series; penis filamentous; genital apertures separated by 5 annuli.
Genus Haemopis.

7. Jaws and teeth present; teeth, 12-16 prs.; colour variable, usually blotched.
Haemopis marmoratis

8. Jaws absent; protruded penis slender and uncoiled; ventral ground colour usually lighter than dorsal.
Haemopis grandis.

9. Jaws absent; protruded penis thick and twisted; ground colour nearly uniform, dark blotches frequently absent or few.

bb. Eyes, 3 or 4 prs.; no jaws; no gastric caeca; genital ducts simple with small atrium produced into a pair of cornua; testes not paired, numerous.
Family Erpobdellidae.

10. Eyes, 3 prs., first pair largest; genital pores separated by 2 annuli; atrial cornua simply curved; longitudinally striped.
Erpobdella punctata

11. Eyes, 4 prs.; genital pores separated by 2 annuli; atrial cornua spirally coiled; colours plain or irregularly blotched.
Nephelopsis obscura.

12. Eyes, 4 prs.; genital pores separated by 3½ annuli.
Dina parva.

BIBLIOGRAPHY

1. Johansson, L. 1909. Die Süsswasserfauna Deutschlands. II. Hirudinea, Egel.

2. Moore, J. Percy, 1901. The Hirudinea of Illinois. Bull. Ill. State Lab. Nat. Hist., 5: 479-546.

3. Ibid 1905. Hirudinea and Oligochaeta collected in the Great Lakes Region. Bull. U.S. Bur. Fish., 25: 153-171.

4. Ibid 1912. The Leeches of Minnesota. Geological and Natural History Survey of Minnesota. Zoological series No. 5.

5. Ibid 1918. The Leeches (Hirudinea). Ward & Whipple, Fresh-water Biology, Chap. XX.

6. Ibid 1922. The Fresh-water Leeches (Hirudinea) of Southern Canada. The Can. Field Nat., 36: 6-10, 37-39.

7. Ibid 1924. The Leeches (Hirudinea) of Lake Nipigon. Univ. Tor. Studies, No. 23.

8. Ryerson, C.G.S., 1915. Notes on the Hirudinea of Georgian Bay. Con. Canadian Biol., 1911-14.