A STUDY OF A LETHAL FACTOR IN THE GROUSE LOCUST,

APOTETTIX EURYCEPHALUS HANCOCK

by

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INTRODUCTION

In 1929, examination of genetic data disclosed the presence of a lethal factor carried in one line of the grouse locusts Apotettix eurycephalus Hancock. The term lethal has been applied to two types of genes that prevent the development of or destroy the individual: those that affect the germ cell are gametic and those that take effect any time after fertilization are zygotic. Although it was evident that this lethal in A. eurycephalus was not gametic because individuals heterozygous for the factor are pro-
duced, it was not known whether fertilization between two lethal-bearing gametes ever occurred, or, if so, at what stage of embryonic development death resulted. The purpose of this investigation has been to determine when and how this lethal factor, when carried homozygously, takes effect.

REVIEW OF LITERATURE

Recessive lethal factors have been studied in Drosophila more than in any other animal or plant. Their presence is usually made evident by missing color patterns and other characteristics, or, if sex-linked, by aberrant sex ratios. The first autosomal lethal which is characterized as streak when heterozygous and lethal when homozygous was discovered in 1915 (Bridges and Morgan, 1919). Other lethals of this type, that is, acting as a dominant characteristic when heterozygous and lethal when homozygous are, on the second chromosome, truncate and star (Morgan and Bridges, 1919), gull and plexate (Morgan, Bridges and Sturtevant, 1925), and curly wing (Ward, 1923), and on the third chromosome, giant, dichaete, delta and minute (Bridges and Morgan, 1923) and beaded (Muller, 1914). Other lethals which are not evident when heterozygous have been located on the second, third and X chromosomes. Some of
these are Lethal-\textsubscript{II\textsubscript{a}} (Bridges and Morgan, 1919) on the second chromosome and Lethal-\textsubscript{III\textsubscript{a}}, Lethal-\textsubscript{III\textsubscript{b}}, Lethal-\textsubscript{III\textsubscript{c}}, etc., (Bridges and Morgan, 1923), on the third chromosome. On the X chromosome are Lethal-1, Lethal-\textsubscript{1\textsubscript{a}}, L\textsubscript{1}, L\textsubscript{r} and L\textsubscript{p} (Morgan, Bridges and Sturtevant, 1925), Lethal-3 (Morgan, 1914b), two other lethals (Morgan, 1914a) and Lethal-4, Lethal-6 and Lethal-7 (Bridges, 1916).

Although the discovery and location of lethal factors in Drosophila has received much attention, little success has been achieved in determining when and how they manifest themselves. Stark (1915) discovered a lethal, designated as \textsubscript{Lsd}, which allowed the homozygous lethal bearing males to emerge from the pupa case and then to die almost immediately upon becoming adults. More attention was given to lethal 7 (Bridges, 1916) which caused the death of the fly at a mature larval stage. While the larvae were still young, one or more intense black spots appeared in their body cavities which became more conspicuous with age until the larvae died. The nature of these black spots or tumors was studied (Stark, 1917). The time at which death took place depended on the stage of development of the tumor. The cause of the tumor, further than that it was initiated by the homozygous lethal was not determined.

Two lethal factors in mice have been studied with more attention to the time at which death occurs. Yellow coat
color in mice was found always to exist in a heterozygous condition (Durham, 1910; Castle and Little, 1910; Little, 1917). The animals homozygous for the yellow factors apparently degenerated in utero (Kirkham, 1916). Ibsen and Steigleder (1917) made an examination of the uteri of yellow mice which had mated to yellow males. They found two types of dead embryos, those which ceased development shortly after implantation and those which died after the thirteenth day. They suggested that those of the first type were the homozygous yellows and that the death of the others was caused by crowding.

Another dominant color pattern in mice, lethal when homozygous, is black-eyed white spotting (Little, 1915). Little found dead embryos in the uteri of black-eyed white females which were pregnant by black-eyed white males. This seemed to indicate, in most cases at least, that the homozygous black-eyed white embryo breaks down after its implantation (Little, 1918). However, in strains of black-eyed white mice, a type of young occurred which died in one to three days after birth (Detlefson, 1922). In appearance they were one-half normal size and white and bloodless. These constituted about one-fourth of the offspring. They were studied (DeAberle, 1925) and found to be anemic. They had a smaller thymus and a larger heart than the normal
offspring. The span of life of these individuals was from a few seconds to eight days. The anemic embryos in utero were easily distinguished from the normals in the same litter. Gowen and Gay (1932) observed that the anemics surviving birth sucked large quantities of milk but were unable to utilize it for their proper nutrition. To keep them alive, it was necessary to inject blood into the peritoneal cavity. However, the injection had to be made every two weeks or oftener and even then, they never overcame the reduced initial size.

Of the four lethals known in cattle, up to 1928, at least three and probably all occurred within the Holstein-Friesian breed. One of these produces almost complete hairlessness. The lethal calves are full-term and normal size but die a few minutes after birth (Mohr and Wriedt, 1928).

Inheritable lethal factors have also been discovered in plants. For example, in maize (Brunson, 1924) a new seedling character is described which segregates as a simple Mendelian recessive. The plants homozygous for the characters are pale green and die in the seedling stage. Another lethal in maize (Mangelsdorf, 1928) reduces the development of the embryo and endosperm and thus renders the seed incapable of germination. In Lolium perenne (Jenkin, 1928a) vigorous, full green plants gave rise, when selfing, to three distinct types of seedlings. One was non-surviving
and died just before or after the appearance of the second leaf. Another lethal caused one-fourth of the plants to die at about the onset of the second leaf stage (Jenkin, 1928b). A recessive lethal in sorghum (Karper, 1930) was found to be responsible for white seedlings.

CHROMOSOME MAP OF APOTETTIX EURYCEPHALUS

Apotettix eurycephalus Hancock has been bred in the greenhouse since 1911. Thirteen dominant color patterns have been brought in from nature, each designated by a letter of the alphabet. These factors have been quite definitely located on one pair of chromosomes (Nabours, 1925), now known to be the first or smallest pair (Nabours, 1931, Robertson, 1931).

\[\begin{array}{c}
B \\
H \\
M \\
Y \\
Ymf \\
\hline
0 & le & 6.9 & 7.4 & 7.43 \\
O & R & W & G & Z0 & TK
\end{array}\]

Fig. 1. Factor map of pair one (the smallest) of the chromosomes of A. eurycephalus.

B, H, M, Y and Ymf are very closely linked or allelo-morphic and are placed at the left end of the diagram. Six and nine-tenths units to the right are located O, R, W and Z. 0 is very closely linked to Z but the data do not show whether to the right or left. Five-tenths of a unit farther
on is T, and close to the right of T are located G and K.
These color patterns, all dominant to the "wild-type" (+/+),
whether heterozygous or homozygous, are easily distinguish-
able. Also each pattern shows up through or modifies every
other pattern (Nabours, 1929).

DISCOVERY OF THE LETHAL

In 1929 when the seven parthenogenetic offspring of a
female of the composition YmfZ0TG/O were recorded, only the
one color pattern, 0/0, was represented. The other expected
pattern, YmfZ0TG/YmfZ0TG, did not appear. At the same time
a sister of the above female, a YmfZ0TG/RK, produced eight
offspring which were all RK/RK. These results suggested
that a lethal factor was carried on the chromosome with Ymf,
Z, 0, T and G.

Following these results by parthenogenesis the other
specimens of this stock were conserved so that in 1930
several test matings were made which definitely indicated
the presence of the lethal. A male YmfZ0TG/RK mated to a
female YmfZ0TG/RK gave as their offspring 5 RK/RK, 13
YmfZ0TG/RK, 1 RTG/RK and 1 YmfZ0TG/Z0TG, the latter two be-
ing crossovers. Another mating of a male and a female both
carrying YmfZ0TG/RK produced a large number of offspring.
These consisted of 76 carrying the pattern complex
YmfZ0TG/RK, 55 with RK/RK and the following crossovers:
3 RTG/RK, 4 ZOTG/RK, 1 YmfRK/RK, 1 YmfZ0TG/Z0TG, and 1 YmfZ0K/RK but no YmfZ0TG/YmfZ0TG. However, the two crossovers recorded as YmfZ0TG/Z0TG might actually have been YmfZ0TG/YmfZ0TG as far as their appearance was concerned, but their numbers would have been larger had there been no lethal present. As it was, they amounted to the number of expected crossovers in this section of the chromosome. Subsequent matings were found which did not have these questioned crossovers. A mating of a YmfZ0K/RK and a YmfZ0/0TG gave 14 YmfZ0/RK, 15 YmfZ0K/0TG, and 11 0TG/RK; and a pair of YmfZ0TG/RK's gave 88 YmfZ0TG/RK, 39 RK/RK, 3 YmfRK/RK and 1 Z0TG/RK. Crossovers resulting from an interchange between Ymf and Z0 were tested for the presence of the lethal. There were found 4 in which the le crossed over with Ymf, 7 where Ymf crossed over without it, 5 where it accompanied Z0 and 3 where Z0 crossed over without it.

These matings indicated the presence of a lethal factor on the chromosome with Ymf, Z, 0, T and G, and its location between the loci of Ymf and Z. This chromosome was then traced back from each of the matings described above to a common progenitor, a male MZ/YmfZ0. This male, MZ/YmfZ0, was from a female MZ/MZ mated to a male +/YmfZ0 which in turn was a crossover from a female carrying Ymf and Z0 on homologous chromosomes. Both of these chromosomes, the one carrying Ymf and the other Z0 were traced back to nature,
Figure 2. The original Ymf chromosome from nature supposedly carried the lethal factor because in testing out the Z8 no evidence for the lethal was found. The lethal was first discovered in the recording of the parthenogenetic offspring of the females 55-128 and 55-129. Mating 57-95 produced the crossovers Ymf/RK in which the lethal crossed over with the Ymf in at least one as indicated in mating 57-216. The female of the mating 57-102 was a crossover in which the lethal remained with the Z8 as the records of the color patterns of the offspring showed.
having been collected at San Antonio, Texas, in 1926. Since at least nine individuals homozygous for Zθ (Zθ/Zθ) and none homozygous for the Ymf (Ymf/Ymf), all descendants of the original specimens, Zθ and Ymf respectively, were recorded, it seems reasonable to conclude that the lethal came in with the latter or mutated along with it in the greenhouse within the five generations intervening between nature and the production of the MZ/YmfZθ male (Figure 2).

**MATERIAL AND METHODS**

*Apotettix eurycephalus* Hancock is a southern form of the grouse locust. It does not hibernate during the winter season and four generations a year may be bred in the greenhouse. The grouse locusts are raised in cages which are made of glass cylinders, about eight inches in diameter and twelve inches in height, with 24-mesh wire screen covers. These are set in bulb pots of sterilized soil which is a mixture of sand and loam. Within the large pot is partly buried a small upturned flower pot. The food consists of algae raised in troughs of running water in the greenhouse or gathered from ponds and streams. Before the grouse locusts are put in the cages, the soil is well soaked and some algae placed around the upturned pot. The cages are watered every day and ground algae is added usually every other day.
Each mating jar contains one pair of adults. A permanent record of the color patterns and parentage of these individuals is kept in a day book. Each mating jar is also labeled so that there is always the least amount of doubt possible as to the characters carried by those mated. The mating jars are inspected daily and any dead grouse locusts are recorded and preserved in alcohol.

The females lay their eggs in the ground, or, in the algae, in bunches of fifteen to thirty-five. The incubation period varies from fifteen to thirty days.

When the lethal-bearing adults, and the normal ones used as controls, had been mated for two weeks, their cages were closely inspected for offspring. When a bunch of eggs hatched, the young were carefully counted and the place from which they had hatched marked. The second day after hatching the offspring were transferred by means of curved tweezers to other cages, usually not over twenty individuals to a cage. Their color patterns were recorded after the third or fourth instar and included in the permanent records.

The batch of eggs from which the hatching had occurred was removed from the ground immediately after the transfer of the young. The eggs were counted under a binocular microscope. Then, by means of dissecting needles, the eggs were teased apart and the unhatched eggs counted. The number of young transferred plus the number unhatched should
equal the total number of eggs counted.

The unhatched eggs procured from the lethal-bearing matings were punctured with a needle and fixed in Bouin's fluid. Later, thirty-five, chosen at random, were placed under a binocular microscope and the shells removed by means of fine, dissecting needles. This was accomplished by breaking off the pointed, or head, end of the egg and gently pushing the embryo out through the opening. The embryos were then cut into transverse serial sections ten microns thick and were studied for the age of development and any deformities which might be discernible.

The unhatched eggs of the controls were not kept. However, another experiment was carried on to help determine the age of development of the homozygous lethal embryos. The breeding cages of the controls were examined for bunches of unhatched eggs. These were taken up and placed in petri dishes containing damp, sandy soil where they could be watched more closely and conveniently. Before the eggs showed the eye-spots as clearly as the lethal eggs did, two eggs were taken from each batch every day, until those left hatched, and were fixed in Bouin's fluid and treated exactly as the lethal eggs. The petri dishes were examined closely for time of hatching as the offspring would not live long in them. Fifty-three slides made from the control embryos were examined to determine on which day before hatching
they were nearest the stage of development of those that
died as a result of the lethal factor.

EXPERIMENTAL DATA

A total of 118 matings of *Apotettix eurycephalus*, both
parents heterozygous for the lethal factor, were made and
studied. Of these matings, 20, approximately a normal
number, failed to produce any offspring. Of those that pro-
duced offspring, 31 furnished no data because of our in-
ability to find the eggs from which the young hatched. Two
difficulties often arise in locating eggs. When the young
hatch they come to the surface of the ground encased in
their amnium which are immediately shed. These white amnium
mark the place where the eggs were deposited. On some kinds
of sandy soil, the amnium are indiscernible. In other cases,
the young hatch so slowly, one at a time instead of all
within a relatively short time, that the amnium of each
left at the surface dries up and darkens before another is
added. The amnium are so small that several are necessary
to identify the place from which the young hatch.

In 12 of the productive matings, it was proved that
one or both of the pair did not carry the lethal factor.
Each mating was tested for this factor in three ways: (1)
by the color pattern, of the offspring, (2) by the presence
of unhatched in a bunch of hatched eggs, and (3) by their
parentage. The presence of a lethal was often questioned because of the possibility of a crossover in the female parent. In every case where one of these criteria indicated the presence or absence of the lethal factor the other two verified the results.

Data were secured from 55 matings, both parents heterozygous for the lethal. These produced 1810 offspring. Of the 2598 eggs counted, 776 were unhatched. The number of offspring should tally with the number of hatched eggs. However, the young sometimes die before they are transferred, and, because they are very small at the time of hatching or because of the sandy nature of the soil, they are difficult to find and count. Mistakes are also possible in counting the eggs. Table I shows the number of offspring, eggs laid and unhatched eggs procured from each mating. The mating number is the number of the day book and the page on which the mating is recorded. These data gave 29.87 per cent of eggs unatched.

There were 33 normal matings, without the lethal, which provided data for the controls. From these, 1378 offspring, 1433 eggs laid and 56 unhatched eggs were counted, as shown in Table II. This gave 3.21 per cent of eggs unhatched in the controls.

Since the control and lethal matings were kept under the same general conditions of temperature, moisture, food
Table I. Lethal Matings

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<th>eggs</th>
<th>No.of:unhatched</th>
<th>Mating:young:eggs</th>
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Total 1810 2598 776
Table II. Control Matings

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<td>1</td>
</tr>
</tbody>
</table>

Total 1378 1433 56

*It is not improbable that matings 62-39 and 62-223 were lethals of another kind. These are to be checked further.
and soil, this 3.21 per cent represents the normal number of unhatched eggs. Therefore, subtracting it from 29.87 per cent leaves 26.66 per cent of eggs unhatched as a result of the homozygous lethal factor. This is very close to the expected result of 25 per cent.

No differences were noted in the time or rate of hatching of the two types of matings. In both, some batches would all emerge within half an hour, the first individual hardly changing color before the last one hatched. At other times they appeared one or a few at a time for a day or more. All the offspring are white with conspicuous dark eyes at the time of hatching.

An examination of the homozygous lethal eggs revealed that most of them contained embryos which had well-developed eye-spots showing through the shell, a condition normal to the eggs of this species a few days before hatching. A few unhatched eggs were found which were totally undeveloped or contained fully developed embryos which had been unable to get out of the shell. The unhatched eggs of the controls were practically all of these two types. The unhatched eggs in any one bunch seemed to be scattered throughout the mass rather than grouped together.

When the shells were removed from the lethal eggs, the embryos showed a body form which was quite far along in development. The mouth-parts and legs were all present but
not so fully developed as those of an embryo ready to hatch.

A study of the serial sections made from the lethals revealed the following facts concerning the development of the embryo:

The dorsal organ or "heart" was present throughout the length of the body.

The stomodaeum and proctodaeum were fully developed. The mesenteron had a thin wall of loosely connected cells and was full of yolk. The wall between the proctodaeum and the mesenteron was dissolved, but the wall between the stomodaeum and mesenteron was only partially dissolved. In most specimens, no yolk cells could be seen in the stomodaeum but some were seen in the proctodaeum. According to Packard (1883), the digestive system becomes a continuous hollow tube, open from mouth to vent "just before hatching".

Tracheal tubes were present, and were especially conspicuous in the jumping legs.

The mesoderm surrounding the mesenteron and in the legs was still largely in a loosely arranged condition with just a small amount of muscle tissue development.

Malpighian tubules were present, opening in to the proctodaeum. The caeca at the anterior end of the mesenteron were still solid diverticula.

Fifty-three slides of serial and sections of embryos taken from the controls were made and studied. Table III
shows the age of the embryos used and the state of development as compared with the lethal embryos. The age was estimated from the day of hatching rather than from the day of oviposition. It is almost impossible to find a female ovipositing, therefore the eggs are taken up when located, and the day of hatching was recorded.

All of the lethal embryos were at the same stage of development. The control embryos which were killed three, two and one day before, and on the day of, hatching showed greater development than the lethal embryos. The increased development of the mesenteron and muscles was especially apparent. I was successful in making serial sections of but two control embryos younger than the lethal ones. These were less developed and therefore contained more yolk and the embryo had not completed blastokinesis. Because of this condition, it was particularly difficult to remove the shell from embryos of this stage. Three embryos four days from hatching and three, five days before, were of approximately the same stage of development as the lethals. These varied in development to a slight degree because the age was reckoned by days and not to the hour. That embryos differing in age of a day or more are at the same stage of development is accounted for by the effect of temperature and moisture on the time of hatching (Bodine, 1925; Uvarov,
<table>
<thead>
<tr>
<th>Mating Number</th>
<th>Days from hatching</th>
<th>Comparison of age to lethals</th>
</tr>
</thead>
<tbody>
<tr>
<td>62-227</td>
<td>0</td>
<td>More developed</td>
</tr>
<tr>
<td>62-227</td>
<td>1</td>
<td>More developed</td>
</tr>
<tr>
<td>62-227</td>
<td>2</td>
<td>More developed</td>
</tr>
<tr>
<td>62-205</td>
<td>2</td>
<td>More developed</td>
</tr>
<tr>
<td>62-192</td>
<td>3</td>
<td>More developed</td>
</tr>
<tr>
<td>62-205</td>
<td>3</td>
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</tr>
<tr>
<td>62-227</td>
<td>3</td>
<td>More developed</td>
</tr>
<tr>
<td>64-87</td>
<td>3</td>
<td>More developed</td>
</tr>
<tr>
<td>64-81</td>
<td>3</td>
<td>More developed</td>
</tr>
<tr>
<td>64-96</td>
<td>3</td>
<td>More developed</td>
</tr>
<tr>
<td>62-227</td>
<td>4</td>
<td>Slightly more developed</td>
</tr>
<tr>
<td>62-192</td>
<td>4</td>
<td>Slightly more developed</td>
</tr>
<tr>
<td>64-81</td>
<td>4</td>
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</tr>
<tr>
<td>64-96</td>
<td>4</td>
<td>More developed</td>
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<tr>
<td>64-87</td>
<td>4</td>
<td>Slightly more developed</td>
</tr>
<tr>
<td>62-192</td>
<td>5</td>
<td>Same stage of development</td>
</tr>
<tr>
<td>62-101</td>
<td>5</td>
<td>Slightly more developed</td>
</tr>
<tr>
<td>64-52</td>
<td>5</td>
<td>More developed</td>
</tr>
<tr>
<td>62-205</td>
<td>6</td>
<td>Slightly more developed</td>
</tr>
<tr>
<td>62-192</td>
<td>6</td>
<td>Less developed</td>
</tr>
</tbody>
</table>
1928). One embryo five days from hatching was more developed than the lethal embryos and just about the same as one embryo three days from hatching.

No apparent physical differences between the control and the lethal embryos was found by a study of the slides.

DISCUSSION

It is evident from the above data that the lethal factor acts as a Mendelian recessive and prevents all homozygotes from completing their embryonic development.

However, the question of whether the lethal embryos developed at the same rate as the other embryos in the same batch of eggs and then stopped development suddenly four or five days before hatching, or whether the development of the lethal embryos was slower during the whole or a part of the embryonic period than the normal embryos is still to be answered.

It would seem that the latter theory is more probable. When the lethal-bearing embryonated eggs were taken up within two days after the mass began to hatch, they were in a good condition for study. If left longer than two days in the soil, the eggs had turned dark and the embryos disintegrated. If the embryos had died four or five days before the rest hatched, it does not seem probable that the dead embryos would still be in condition for study. Also, my
success in killing and fixing the embryos in Bouin's fluid suggested that the tissue was still living. That these supposedly more slowly developing embryos would have eventually hatched does not seem probable because of the disintegration of the eggs left in the soil, and because, when the eggs were not located and molested, there was no later hatching of any embryos carrying the missing color pattern combinations.

There remains then two problems of research in this project: Why does the development of the lethal embryos take place more slowly, if it does, and why do they die when the others hatch?

SUMMARY

1. Fifty-five matings of grouse locusts heterozygous for a lethal factor gave data; and the eggs were inspected for unhatched ones. The shells were removed from thirty-five of the unhatched eggs and the embryos were made into serial sections and compared with slides of control embryos.

2. The lethal matings gave a ratio of 1810 hatched eggs to 776 unhatched eggs. The controls gave 1378 hatched to 56 unhatched. Allowing for 3.21 per cent of unhatched eggs in the controls, this gave a total of 26.66 per cent of unhatched eggs due to the lethal factor.
3. The serial sections were examined for differences in stage of development and structural characteristics. No physical deformities or irregularities were found in those dying as a result of the lethal. The stage of development of the lethal embryos at the time the egg mass hatched corresponded to the development of the control embryos which were four or five days from hatching.

4. The lethal factor is zygotic in effect. It manifests itself in the embryonic period and prevents the embryo homozygous for it from developing beyond the stage of that of a normal embryo four or five days before hatching.

5. It still remains to be discovered: first, whether the embryo ceases development suddenly four or five days before hatching or whether the development is merely retarded and then ceases entirely about the time the others hatch; and second, what the physical or physiological results of the lethal are which cause death.

ACKNOWLEDGMENTS

Dr. Robert K. Nabours, who discovered the lethal, has furnished the specimens and cooperated in the collection and interpretation of these data, and criticized the manuscript. Dr. Roger C. Smith has aided in the study of the slides of the embryos.
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