SEX DETERMINATION IN *ANTHOTHIRIPS VERBASCI*

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The mullein thrips is a species that was formerly supposed to reproduce only sexually. Both sexes are abundant; fully formed spermatozoa are produced by the male; and copulation may frequently be observed in nature. Two years ago, however, it was discovered (SHULL 1915) that virgin females also produced offspring. At the time of publication of the paper cited the sex of such parthenogenetically produced offspring was unknown. It could not be stated, therefore, whether the parthenogenesis of *Anthothrips* was of the aphid type, in which both sexes develop from parthenogenetic eggs; or of the honey-bee type, in which fertilized eggs produce females, unfertilized eggs males; or of a third type unlike any species now known.

The experiments here described were designed to solve the problem of the relation of parthenogenesis to sex in this species.

VIRGIN FEMALES PRODUCE ONLY MALES

The experiments that showed that virgin females produce only sons were of two kinds. These two kinds differed only in the time elapsing between the beginning of the experiment and the removal of the parents from the host plant. In some experiments the parents were removed before the first offspring became adult, in others not until adult offspring were present.

Experiments in which the parents were removed while the oldest offspring were still immature

Virgin females were procured by rearing pupae in isolation until they emerged as adults. The sex was ascertained by placing the live insect in a drop of water under a supported cover glass, and examining with a microscope. Only the females were retained. These were placed on young mullein plants that had been reared from seed under cover, and

were protected by lantern globes closed at the top with closely woven cloth. In some experiments only one female was placed on a single plant, in others a number of females were put together on the same plant. All these females were removed before the first offspring became adult. The offspring subsequently removed are recorded in table 1.

TABLE I

A record of the sex of the offspring of virgin females of Anthothrips verbasci, in experiments in which the parents were removed before any of the offspring became adult.

Number of experiment	Number of females used as parents	Date of beginning of experiment	Date of removal of parents	Offspring	
				Males	Females
93	1	Aug. 28, 1914	Apr. 12	1	0
95	2	Aug. 30, 1914	Apr. 12	18	0
99	5	Sept. 11, 1914	Dec. 5	63	0
100	7	Sept. 14, 1914	Dec. 5	9	0
101	7	Sept. 16, 1914	Apr. 12	8	0
102	9	Sept. 16, 1914	Dec. 5	22	0
129	1	July 5, 1915	July 29	6	0
131	3	July 9, 1915	July 29	68	0
132	3	July 9, 1915	July 29	34	0
133	3	July 12, 1915	July 29	48	0
134	5	July 12, 1915	July 29	60	0
135	5	July 12, 1915	July 29	57	0
145	4	July 14, 1915	July 30	39	0
146	2	July 17, 1915	July 30	18	0
147	5	July 19, 1915	July 30	79	0
149	7	July 19, 1915	July 30	32	0
150	7	July 19, 1915	July 30	53	0
151	8	July 22, 1915	July 30	32	0
165	3	July 29, 1915	Sept. 21	38	0
170	5	July 29, 1915	Sept. 22	3	0
Totals	92			688	0

There is abundant proof in these experiments that unfertilized eggs produce males.

Experiments in which the parents were not removed until the first offspring were adult

These experiments were conducted in every way like those recorded in table 1, except that the parents remained on the host plant until some of the offspring were adult. When adults were subsequently removed from the plant, they may or may not have included the parents, depending on

whether the latter survived. The sex of the thrips removed from such plants is stated in table 2.

TABLE 2

A record of the sex of individuals of Anthothrips verbasci taken from plants on which virgin females had previously been placed. The virgin females were left on the plant until some of the offspring were adult, and may be included in this list with the offspring.

Number of experiment	Number of females used as parents	Date of beginning of experiment	Date of removal of parents	Offspring	
				Males	Females
102a	9	Dec. 5, 1915	June 9	15	3
148	5	July 19, 1915	Sept. 20	61	1
152	5	July 24, 1915	Sept. 20	19	3
153	5	July 24, 1915	Sept. 20	45	3
154	5	July 28, 1915	Sept. 20	47	5
155	6	July 28, 1915	Sept. 20	19	5
167	3	July 29, 1915	Sept. 21	28	2
168	5	July 29, 1915	Sept. 21	85	1
169	5	July 29, 1915	Sept. 22	61	2
177	4	July 30, 1915	Sept. 22	63	1
179	7	July 30, 1915	Sept. 23	12	2
Totals	59			455	28

While some females appear among the adults obtained from these plants, the number of females is in no case greater than the number of female parents with which the experiment was started. It is to be assumed, then, in harmony with the experiments of table 1, that the males in table 2 are the offspring, the females are the mothers.

From the above experiments, in both tables, it is sufficiently well established that unfertilized eggs produce only males.

FEMALES THAT MATE MAY PRODUCE BOTH SEXES

In a series of experiments females were either induced to mate in captivity, or were observed to mate in nature, and were then transferred to mulleins in the greenhouse and kept under cover. In each experiment the female alone was placed on the host plant, and she was removed before any of her offspring became adult. These offspring, on becoming adult, were removed and their sex ascertained. In some experiments they were collected from the host plant only once, in others several times. But in every experiment examination of the offspring ceased before any of their offspring (grandchildren of the original female) could have become adult. The records are certainly, therefore, those of the imme-

diate children only. In some experiments both daughters and sons were produced, in others only sons. Tables 3 and 4 show the sex of the offspring in these experiments, grouped for convenience according to the results.

TABLE 3

A record of the sex of the offspring of females of Anthothrips verbasci which were known to have mated, and subsequently produced both daughters and sons.

Number of experiment	Date of beginning experiment	Offspring	
		Males	Females
97	Sept. 3, 1914	18	2
117	July 5, 1915	52	4
119	July 5, 1915	6	2
120	July 5, 1915	33	3
125	July 5, 1915	5	3
138	July 12, 1915	5	2
139	July 12, 1915	51	7
140	July 12, 1915	2	1
143	July 12, 1915	23	6
144	July 12, 1915	32	7
159	July 28, 1915	31	8
Totals		258	45

TABLE 4

A record of the sex of the offspring of females of Anthothrips verbasci which were known to have mated, and subsequently produced only sons.

Number of experiment	Date of beginning experiment	Offspring	
		Males	Females
118	July 5, 1915	2	0
126	July 5, 1915	3	0
137	July 12, 1915	39	0
141	July 12, 1915	16	0
142	July 12, 1915	43	0
Totals		103	0

In harmony with the results shown in table 1, it is to be assumed that the males in table 4 were produced from unfertilized eggs, notwithstanding their mothers had mated. The occurrence of females among the offspring in table 3 and the production of nothing but males in table 1 indicate that fertilized eggs yield females, at least in part. The paucity of females may be partly accounted for by the fact that the females

mated only once, whereas in nature they may be observed to mate repeatedly.

Support for the conclusion that fertilized eggs yield females, at least in part, and unfertilized eggs always males, is found in the offspring of mixed lots of males and females. Two such lots were maintained for a time to provide a stock of thrips for use in experiments, and to ascertain what would be the sex ratio in the greenhouse under conditions as favorable as possible to repeated mating. Table 5 gives the sex of the adults collected from these two plants at intervals, each collection including all the adults present on the date named.

TABLE 5

*Showing the sex ratio of the offspring produced by a mixed lot of males and females of *Anthothrips verbasci* under conditions favorable to repeated mating.*

Number of experiment	Date of beginning experiment	Dates of collecting adult offspring	Offspring	
			Males	Females
203	June 28, 1916	Sept. 9, 1916	6	0
		Oct. 4, 1916	18	8
		Oct. 23, 1916	16	0
204	June 28, 1916	Sept. 9, 1916	1	2
		Sept. 30, 1916	0	3
		Oct. 15, 1916	0	2
		Oct. 24, 1916	25	43
		Nov. 23, 1916	15	7

These experiments are cited here as showing that when males are present, females appear among the offspring. The sex ratio and its significance is referred to elsewhere.

ALTERATION OF THE SEX RATIO IN FAMILIES OF FEMALES THAT MATE ONCE

From certain theoretical considerations discussed below, it was important to ascertain the sex ratio of the offspring of a female that had mated, and whether that sex ratio is altered if subsequent matings are prevented. Females that were known to have mated were placed alone upon mulleins. Before any of their offspring became adult, they were transferred to new plants. From this second plant the offspring were removed when they became adult, care being taken not to collect so late that any of the grandchildren might be included. In table 6 the sex ratio of the collection from each plant in these experiments is given.

The occurrence of females on the first plant of each experiment, and their absence from the second plant in most cases, is explained on the

TABLE 6

A record of the sex ratio of the offspring of single females of Anthothrips verbasci, known to have mated, the offspring being collected at intervals as they became adult.

Number of experiment	Date of beginning experiment	Dates of collecting adult offspring	Offspring	
			Males	Females
117	July 5, 1915	Sept. 19,* 1915	37	4
		Sept. 21, 1915	15	0
139	July 12, 1915	Sept. 20,* 1915	26	7
		Sept. 22, 1915	25	0
143	July 12, 1915	Sept. 20,* 1915	7	6
		Sept. 22, 1915	15	0
		Oct. 14, 1915	1	0
144	July 12, 1915	Sept. 20,* 1915	21	6
		Sept. 22, 1915	11	1
159	July 28, 1915	Sept. 21,* 1915	22	8
		Oct. 14, 1915	9	0

* Must have been mature much earlier. Were on the first plant to which the female was transferred.

assumption that the spermatozoa were all allowed to fertilize eggs at first, and that subsequent eggs were unfertilized.

Another experiment not included in table 6 because performed in a slightly different way, bears upon the same theoretical point, and is here recorded separately. In experiment 94, a virgin female and a male were placed together on a plant December 16, 1914. Copulation was not witnessed, but the results indicate that mating occurred. The first offspring became adult shortly before June 18, 1915, on which date all adults were removed. These proved to be 9 males and 27 females, and they may or may not have included the original parents. On June 29 another collection of adult offspring was made, comprising 53 males and 60 females. Further collections were made, but since the grandchildren may have had time to mature before these later lots were collected, the sex ratio of the later collections is of no value in this connection. In the figures given it is worthy of note that the females are in the majority, distinctly so in the first collection. The importance of this fact is indicated elsewhere.

DISCUSSION

From the experiments described it was early established that unferti-

lized eggs of *Anthothrips verbasci* produce only males. I also conclude that fertilized eggs produce only females, but was not forced to this conclusion until consideration of the above experiments left no other assumption probable. By analogy with the honey-bee, it was to be expected that, if parthenogenetic eggs yielded males, fertilized eggs would yield only females. But another analogy with the honey-bee, the degeneration of the male-producing spermatozoa, could not be established. The close relationship of the thrips to the Hemiptera on the one hand and the Orthoptera on the other, lead one to expect two types of spermatozoa, respectively male-producing and female-producing, in the thrips. Moreover, casual observations made upon poorly fixed material showed, in some cell divisions, what appeared to be a lagging chromosome, though I have not verified this observation on better material. However, if two types of spermatozoa are produced, analogy with the honey-bee requires the male-producing type to degenerate. This degeneration I have been unable to see in sections, though the minuteness of the object may account for my failure.

If sex in the thrips is not determined in the same way as in the honey-bee, there seemed but one probable alternative. It appeared possible that unfertilized eggs gave rise to males, and fertilized eggs to both males and females, depending on which type of spermatozoön fertilized the egg. The fact that no case of this kind is yet on record did not warrant the rejection of the hypothesis without consideration.

In the absence of any cytological evidence, the major part of the experiments described above were performed to test the correctness of the two hypotheses just outlined.

If the sex of thrips is determined in the same way as that of the honey-bee, the sex ratio may be anything. If, on the other hand, fertilized eggs yield males as well as females, in equal numbers, because there are two types of spermatozoa, the males must always predominate; for, to the fifty percent of males hatching from fertilized eggs must be added those developing from unfertilized eggs. The latter view also requires that there be two kinds of male, one from parthenogenetic and one from fertilized eggs.

The sex ratio among the offspring of impregnated females, as found in the greenhouse, usually showed a preponderance of males. Tables 3, 5, and 6 afford abundant illustrations. Certain exceptions, however, are crucial. Experiment 94 showed that a female which had every opportunity to mate produced, among her first offspring, 27 females and 9 males. A later collection comprised 60 females and 53 males. Like-

wise, in experiment 204 (table 5), which was started with a mixed lot of males and females, the first four collections of offspring showed a preponderance of females. The marked excess of females in these two cases seems to exclude the hypothesis that males arise from all parthenogenetic and half the fertilized eggs, because by that hypothesis there could never be, except by rare chance, a large majority of females. Furthermore, no evidence has been obtained that there are males of two kinds.

It seems necessary to conclude, therefore, that sex in this species of thrips is determined as in the honey-bee. Unfertilized eggs always produce males, fertilized eggs always females. Moreover, there is, as in the honey-bee, some means of withholding the spermatozoa, so that unfertilized eggs may be laid even while spermatozoa are present. Evidence that this is true is found in experiment 144 (table 6). The offspring produced on the first plant, which were collected September 20, included a large proportion of males. Yet, from the second plant, to which the mother was transferred July 30, one female and eleven males were taken September 22. The one female was taken as a pupa on that date and was allowed to emerge as an adult in the laboratory. The spermatozoon which fertilized the egg from which she developed must have been withheld while at least 21 earlier eggs were laid without fertilization.

This statement of the method of sex determination in thrips is made for only one species, *Anthothrips verbasci*. It is not improbable that it is true for some other species; but it can hardly be applied to species in which there are very few males, and certainly not to those in which there are no males. In *Thrips tabaci*, for example, males are rare (SHULL 1914); likewise in *Anaphothrips striatus* in certain regions (HINDS 1902). In these species, and others like them in this respect, rarity of males at any given time should, if sex were determined as in the mullein thrips, result in the parthenogenetic development of most of the eggs laid; and hence in an abundance of males in the next generation. The long periods of time over which the sex ratio of such species has been observed, preclude the possibility of a fluctuation as extreme as that mentioned. In *Thrips tabaci*, therefore, and in *Anaphothrips striatus* in regions where males are rare, the occasional males must arise through some change in the eggs (perhaps in their maturation), as in the aphids and phylloxerans.

That *Anthothrips verbasci* is not, however, the only thrips in which sex is dependent upon fertilization, is indicated by peculiarities of the sex ratio which were formerly very puzzling. In *Chirothrips manicatus*,

a species living on timothy, bluegrass and other grasses, the sex ratio has been observed (SHULL 1914) to fluctuate enormously within short periods of time. At Douglas Lake, Michigan, this species was observed at intervals in a small island of timothy in a thicket of bushes and small trees. As *Chirothrips* is not a ready flier, and shows little inclination even to crawl, the phenomenon about to be described can hardly have been due to migration. In this island of timothy the adult specimens collected were at first mostly females. Then adults practically disappeared for a time. A week or two later adults were again fairly abundant, but nearly all were males. A similar change in the sex ratio has since been observed in the outskirts of Ann Arbor, and I am told by Mr. C. B. WILLIAMS that he has seen the same occurrence in England. In the light of the experiments on *Anthothrips verbasci*, I think we may interpret the peculiar changes in the sex ratio of *Chirothrips manicatus*. The mothers of the adults collected were probably impregnated, and the first offspring were mostly females. For some reason mating did not occur again, and subsequent eggs developed parthenogenetically, producing males. *Chirothrips* is not as easy to rear under guarded conditions as *Anthothrips*, and experiments intended to verify or refute the above hypothesis failed. The only other explanation of the peculiarities of the sex ratio in *Chirothrips* that seems plausible is that perhaps the males require longer to develop than the females. From my experience with other insects I should expect just the reverse. I assume, therefore, that sex in *Chirothrips manicatus* is probably dependent on fertilization, just as in *Anthothrips verbasci*.

One more question is raised, but not answered, by the results of the experiments with *Anthothrips*. Why are the females more abundant than males in nature, whereas in the insectary the males have been found to be in the majority? It may be that the conditions of the greenhouse are not favorable to repeated copulation. Or the males may be short-lived, a fact which would not be detected in the experiments, since adults were usually still young when collected. Or the males may migrate more readily than the females. These hypotheses could readily be tested by experiment.

LITERATURE CITED

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