FERTILITY AND TOLERATION OF LOW TEMPERATURE IN *EUCHALCIDIA CARYOBORI*, HANNA (HYMENOPTERA, CHALCIDINAE).

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During my work on the life-history of the Chalcid, *Euchalcidia caryobori*, Hanna (Hanna 1933), which parasitises the pupae and the late larval stages of the Bruchid, *Caryoborus pallidus*, Oliv., infesting senna pods, great difficulty was experienced in obtaining the material. Infested senna pods arrive in London from the Sudan between June and August. Any pods arriving after or before that are not usually infested to any considerable extent. To guard against this lack of material, a large supply of parasitised and unparasitised pupae of the Bruchid was obtained when available by sifting the senna pods and keeping them in large glass jars at a constant temperature of 27°C. The Chalcid soon emerges and deposits its eggs through the cocoon on the unparasitised pupae of the Bruchid and feeds on peeled sultanas placed on a cardboard tray on top of the material and moistened every day. The sultanas were changed every three days to avoid fermentation.

The parasitised pupae of the Bruchid containing only the early stages of the pupae of the Chalcid were always picked out of the culture every two days. It is possible to see through the cocoons by placing them on a plate of glass standing over a strong light. They were then placed in glass tubes in the laboratory with a temperature ranging roughly from 15 to 23°C. This low temperature was enough to retard the growth of the pupae for a considerable time. If, on the other hand, the imago of the Chalcid was required at any time, some of these pupae could be transferred to a constant temperature of 27°C., at which they soon emerge.

For the purpose of working out the number of eggs laid by each female and the difference in the length of the life-history of the male and female, 53 pairs were obtained by transferring some of the above-mentioned parasitised Bruchid pupae from the laboratory, where they had been kept for 15 days, to a constant temperature of 27°C. After emergence each pair was placed in a glass tube with a piece of moistened sultana and five pupae of the Bruchid, which were dissected daily and replaced by fresh material. The eggs obtained were reared exposed on the host in block watch-glasses to observe their development. Out of 53 pairs 39 lived for the normal period. The rest died without laying the full complement of eggs, probably because of their antennae being cut off or because they became sticky with syrup from the sultanas. The average number of eggs laid was 88, with a maximum of 181. Two individuals did not lay eggs at all. Altogether *132 males and 18 females were obtained, which shows an overwhelming preponderance of males. In ordinary circumstances (*i.e.*, continuously at 27°C.) the males and females are equal in numbers. This result was surprising and led me to suppose that there might be some factor at work resulting in one or more of the following : (1) The non-formation of sperm ; (2) the failure of the sperm to reach the egg ; (3) the failure of the fertilisation process itself. But whatever that factor was, it did not greatly affect the egg-laying capacity of the females, for the average number of eggs laid was very little below the normal average, which is 102.

To find out whether this biological defect lay in the males or in the females, 15 female pupae were kept in the laboratory for 15 days immediately after pupation, and after emergence each was placed with a male bred from egg to adult in a constant

* The smallness of the number reared is explained later (p. 317).

temperature of 27°C., and then the same procedure as in the above-mentioned experiment was followed. The number of females produced was 34, that of the males 39. It seems evident, therefore, that nothing hindered the fertilisation of the eggs. It was therefore thought that the males were defective in the first experiment, and this would account for the great preponderance of males over females in my breeding work, since unfertilised eggs always produce males.

It was thought desirable to investigate the factor which so acted on the males. At the outset it looked as if the inbreeding of the individuals in the culture for two or more generations, together with the artificial conditions under which they lived, was the chief factor. Testing the egg-laying capacity of normal females and the fertility of the males which were in the culture and had always been kept at a constant temperature of 27°C. was enough to establish or eliminate the importance of that factor. Thus 56 pairs, just emerging, were taken and the number of eggs laid by each female was recorded. The average number of eggs deposited was 98, which is the normal number of eggs laid, and the maximum 220. Four females did not lay any eggs. The number of males obtained was 136, and 143 females, thus showing that inbreeding was not the real cause.

It remained to study the possible effect of low temperature on the pupae. If they were kept in the laboratory for a longer time than 15 days, would it also affect the egg-laying capacity of the females ? Furthermore, would it be possible to reach a point where the emerging females would not lay any eggs at all ? For this purpose 37 females that had been kept in the laboratory as early pupae for 36 days were obtained and each placed in a tube with a male reared from egg to adult in a constant temperature of 27°C. The average number of eggs laid was 7, the maximum 32. Fourteen females did not lay eggs at all ; 18 males and 10 females were obtained, and although there was a majority of males over females, yet the number was not large enough to draw any conclusion as to the defect of the males.

Again, 22 pupae were kept in the laboratory 50 days, 2 females only laid eggs, 1 and 37 respectively, and 20 females died before laying any eggs.

It is evident, therefore, that temperature was the main factor, acting first on the males and later on the females.

The above-mentioned preliminary experiments were carried out on the early pupae to define the factor, and then other experiments under more controlled temperature conditions were devised. This was obtained by an electric incubator ; but although its temperature was fairly constant at 16°C., it occasionally gave a range from between 13° to 19°C.

In the following experiments the eggs and larval stages were reared exposed on the host in block watch-glasses at a constant temperature of 27°C., and the pupae were transferred to the incubator immediately after their metamorphosis from the larvae. They were divided into three groups, each being subjected to the low temperature of the incubator for a certain period, after which they were transferred to a constant temperature of 27°C. until emergence. Then each pair was kept in a separate glass tube with a piece of sultana and 5 pupae of the Bruchid, which were dissected and replaced every day. The glass tubes were always placed in a constant temperature of 27°C. in a large glass jar with an air-tight stopper to prevent the drying-up of the sultanas. The eggs obtained were reared exposed on the host in block watch-glasses.

In the *first group* 25 female and 25 male pupae were subjected to a temperature of about 16°C. for 10 days. The average number of eggs laid was 81, which is a little below the average normal of 102 ; the maximum 127. The eggs belonging to each female were kept in a separate watch-glass so that it would be possible to recognise which females had been fertilised. Altogether 122 males and 25 females were produced. The latter were obtained from 7 males, and if we take into account the 2 females

that did not lay any eggs, we can gather that 7 out of 23 females were successfully fertilised, or in other words about 70 per cent. of the males were sterile.

In the *second group* 22 female and 22 male pupae were kept at 16°C. for 25 days. Only 8 females laid eggs; the average number of eggs was 6, the maximum 54. Fourteen, or 63·5 per cent., of the females did not lay any eggs. Fifteen individuals were obtained from these eggs, all males.

Because the number of eggs laid by this group was very small, it was not possible to determine the fertility of the males in the same experiment, and thus it was thought desirable to test the fertility of the males with females that had been bred throughout in a constant temperature of 27°C. Thus 20 male pupae were subjected to a temperature of about 16°C. for 25 days and, after emergence in a constant temperature of 27°C., each was kept with a female bred in the latter temperature. The eggs of each female were again kept separate. The number of individuals produced was 108 males and 7 females, the latter produced by two mothers. The sterile males were therefore 90 per cent.

In the *third group* 32 female pupae were subjected to the low temperature for a still longer time, 40 days. Three females only laid 3, 4, and 51 eggs, respectively, 29 (or about 90·5 per cent.) did not lay any eggs.

To test the fertility of the males of the latter group 20 of them were each placed with a female bred throughout in a constant temperature of 27°C.; 116 individuals were produced, all being males (sterility 100 per cent.).

It will be noticed that the number of individuals produced from these experiments was very small, as a great number of eggs failed to hatch because they were reared exposed; moreover, owing to the occurrence of superparasitism among the larvae, a great many of them were attacked by fellow larvae; if two or more happened to be on the same host, one larva only survived.

It may be argued that exposure (*i.e.*, rearing on host-pupae extracted from the cocoon) may have a differential effect on the eggs in such a way that it would affect the eggs destined to be females, and this may account for the preponderance of males over females. But in the two experiments mentioned above in which the fertility of the males was tested, the eggs were also exposed, and yet the number of males and females were equal.

Effect of Low Temperature on the Size of the Testes.

The testes of the males belonging to the third group (pupae kept cool for 40 days) were larger than those of normal males. This may have been due to the passage of the spermatozoa from the testes to the vesicula seminalis in the normal males, while the absence or reduction of spermatozoa in the third group may account for the large size of their testes.

Effect of Low Temperature on the Larvae.

Sixty last stage larvae were exposed to the low temperature of the incubator for 55 days; 5 of them metamorphosed into pupae and the rest were in such a dormant state that it was very difficult to know whether they were dead or alive. Then they were placed separately in a constant temperature of 27°C. After emergence, each couple was kept in a glass tube with pupae of the host and fed as in the previous experiments. The average number of eggs laid was 90, which is very little below the normal average. The number of males produced was 69 and that of the females 62; therefore the fertility of the adults was not affected when their larvae were subjected to the low temperature for a very long time, and it seems likely that under natural conditions the species passes the winter as a larva. This possibility is supported by the fact that some live larvae were collected from London docks

in February after passing the English winter, while all the pupae and the adults were dead.

According to Dr. Hurst, the chief meteorologist of Egypt, the average temperature during the winter in the region of Port Sudan, from which the senna was shipped, is 22°C., and it seems that the insect escapes the effect of that low temperature by hibernating as a larva.

Effect of Low Temperature on the Coloration of the Adult.

It was noticed that the colour of the adult becomes darker under the influence of low temperature. The antennae, tarsi, and tegulae, especially of the female, which are very pale brown in normal specimens, becomes almost black. This change of colour has been noticed by many authors in different families of Hymenoptera.

Effect of Low Temperature on the Reproductive Organs.

The Female.

The microscopic examination of the ovaries of females of different ages belonging to the second and third groups (fig. 1), as compared with the newly emerged normal ones (fig. 2), shows that low temperature has a differential effect on the ovarioles. In its extreme effect we find the six ovarioles shrunk into very short tubules; in this case the female would be completely sterile. Some have one or more ovarioles on one or both sides shrunken or degenerate, while the rest contain small nutritive chambers alternating with very small immature eggs. In some cases this condition was found even in females 47 days old, the average longevity of the normal female

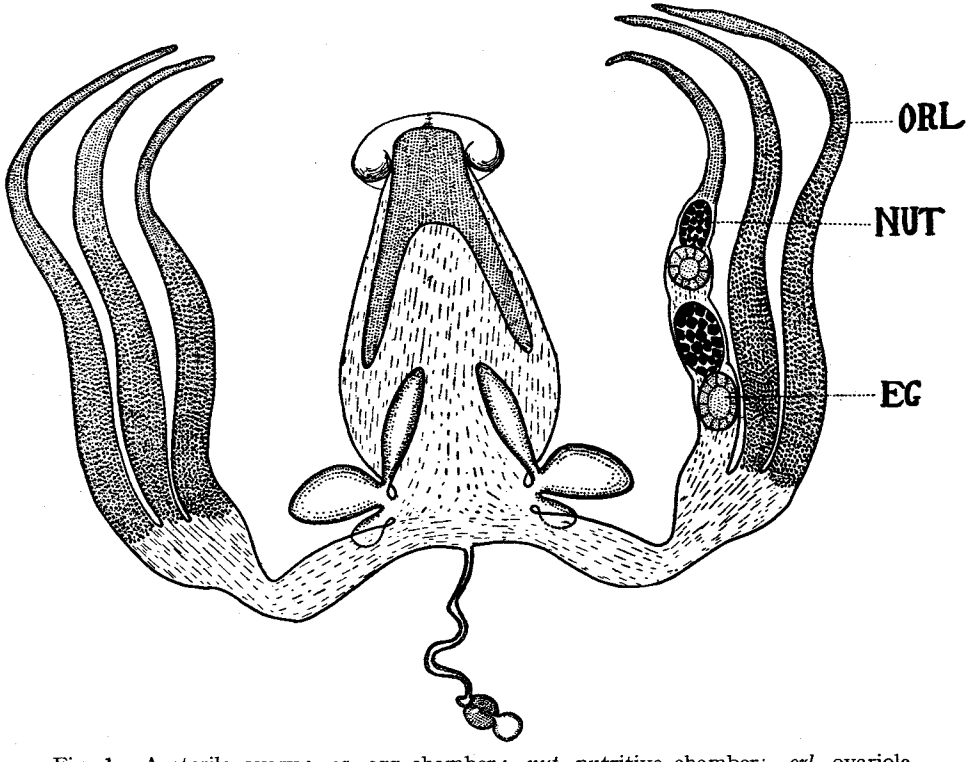

Fig. 1. A sterile ovary: *eg*, egg chamber; *nut*, nutritive chamber; *orl*, ovariole.

being 51 days. In some preparations, especially of young females, we find the ovarioles consisting of long slender tubules similar to those of the pupae without any differentiation into egg- and nutritive-chambers, and it seems probable that these females, had they lived, would have laid eggs later. On the other hand, some ovarioles were found to contain mature eggs but not so many as the normal ones. Females having such ovarioles would lay an egg or two occasionally.

From this, it seems that low temperature continued over a long time may cause either the retardation of the growth of the eggs, or, in its extreme effect, the malformation of one or more ovarioles.

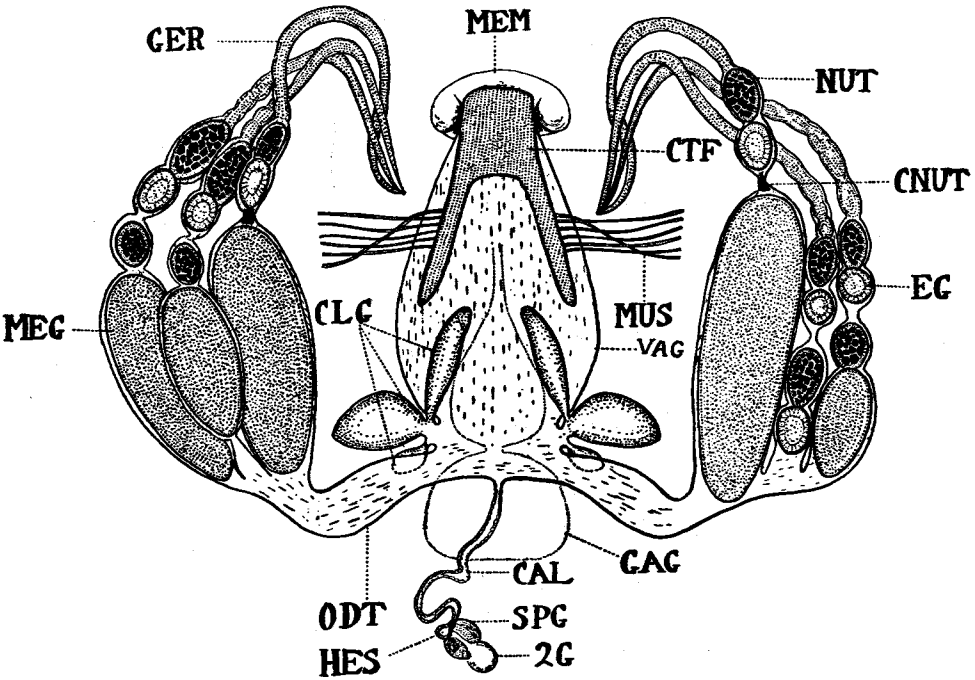

Fig. 2. Ovary of a newly emerged female : *cal*, canal ; *clg*, colleterial glands ; *eg*, egg ; *gag*, 2nd abdominal ganglion ; *cnut*, collapsing nutritive chamber ; *ctf*, chitinous fork ; *2g*, 2nd gland of spermatheca ; *ger*, germarium ; *hes*, head of spermatheca ; *meg*, mature egg ; *mem*, membrane ; *mus*, muscles ; *nut*, nutritive chamber ; *odt*, oviduct ; *spg*, spermatheca ; *vag*, vagina.

The Male.

From the microscopic study of the vesiculae seminales, it seems very difficult to draw a sharp line between sterile and fertile individuals by the criterion of the presence or absence of the sperm, especially in the first and second groups.

It is remarkable that some males had in their vesiculae seminales what appeared to be scattered spermatozoa and yet failed to fertilise females, though some of them were seen to copulate. It may be noted that copulation cannot be seen very frequently, as the females do not copulate more than once.

The vesiculae seminales of the males belonging to the third group were examined in males from 1 to 10 days old, but seemed to be empty.

Five testes of each of the three groups and also five testes of males reared at a constant temperature of 27°C. were cut in paraffin, transversely, with a thickness of 5 μ, and stained in Heidenhain's iron haematoxylin. All males used were 2 days

old and the sections chosen for examination were as near the middle region as possible.

Testes of the males reared at 27°C. contained a great number of spermatozoa (fig. 3), some specimens being full of them, while the premetamorphic cells (which do not exhibit any trace of metamorphosing sperm) were very few, sometimes absent.

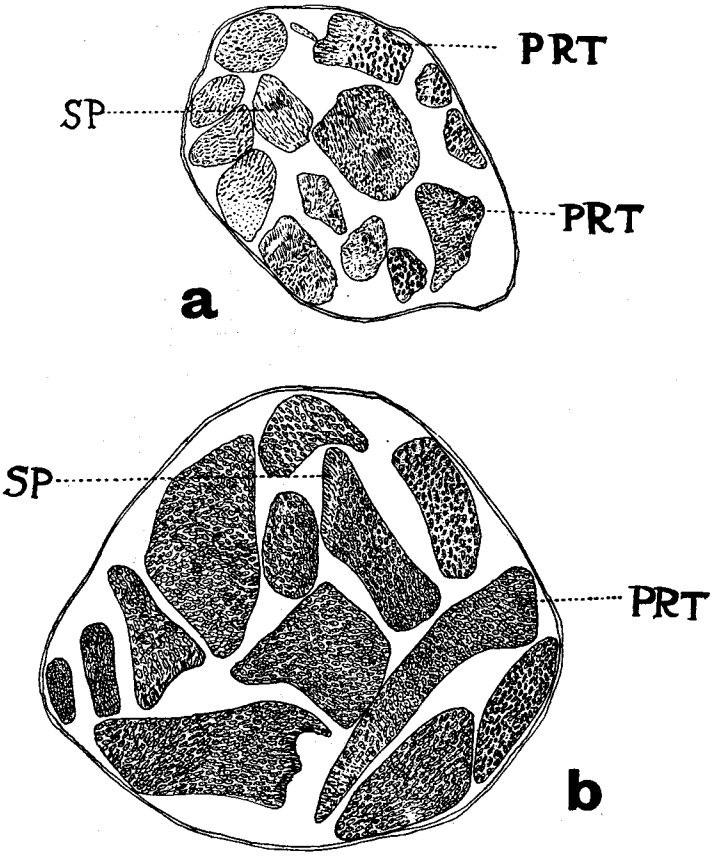

Fig. 3. *a*, transverse section of a testis of a normal male (2 days old) ; *b*, transverse section of a testis of an adult male of the third group, two days old (pupa kept 40 days at 16°C.) *prt*, premetamorphic cells ; *sp*, sperm.

The spermatozoa in the testes of the first group were fewer than in the normal group, while premetamorphic cells were present, but not frequent.

In the second group, the spermatozoa were few, but the premetamorphic cells were abundant.

The spermatozoa in the third group were very few indeed, sometimes absent, and the testes were almost full of premetamorphic cells (fig. 3, *prt*).

In the second and the third group distinct signs of degeneration were shown by the premetamorphic cells.

From this it will be gathered, that low temperature causes, at least, the retardation of spermatogenesis. In its extreme effect it may cause the degeneration of the premetamorphic cells.

Discussion.

Exact experimental data on the influence of low temperature on the fertility of insects are not very extensive.

Sikora (1915) found that the louse, *Pediculus vestimenti*, L., did not lay any eggs at a temperature lower than 25°C. Pospelov's work (1916, 1926) on the maturation of the gonads of *Locusta migratoria*, L., shows that they mature within a month at a temperature of 35°–38°C., while individuals kept at a temperature of 20°–30°C. do not lay any eggs. Voelkel (1924) kept the males of the beetle, *Trogoderma granarium*, Everts, at a temperature from −8° to −10°C. for 3–4 hours and found that it did not affect their fertility. Males kept at −8°C. for 30 hours were sterile. Mayne (1926) showed that the mosquito, *Anopheles quadrimaculatus*, Say, does not lay any eggs between 4·4° to 12·2°C.

The result of the experiments described in this work shows that the tissues of the testes are more sensitive to low temperatures than the tissues of the ovaries; for, after exposing the pupae of both the male and the female to a low temperature (16°C.) for 10 days, the females still layed the normal number of eggs, while 70 per cent. of the males were sterile. These results seem to agree with those obtained by Norris (1933) in her work on the sterility of *Ephestia kühniella*, Z., at high temperatures. She found that the sterility was due to some abnormality on the part of the males only.

Young & Plough reared *Drosophila* at a temperature of 32°C. and found that 96 per cent. of the males were sterile, but only 50 per cent. of the females.

Raichoudhury's work on the retardation of spermatogenesis in *Ephestia kühniella*, Z. (in press), at high temperatures (27° and 30°C.), and Young & Plough's on *Drosophila* (also at high temperatures), show that the testes of sterile individuals contain some scattered spermatozoa, which is also the case in the testes of my sterile groups. It appears that those spermatozoa which are produced must be in some way abnormal, but this question has not been investigated.

Acknowledgments.

My thanks are due to Professor J. W. Munro under whose direction this work was carried out. I am also very much indebted to Mr. O. W. Richards, Mrs. O. W. Richards, Mr. H. R. Hewer and Dr. D. P. Raichoudhury, for valuable advice and help.

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Reprinted from the BULLETIN OF ENTOMOLOGICAL RESEARCH, VOL. **26**, Part 3, *September*, 1935.