REVERSAL OF SEX PRODUCTION IN MICROMALTHUS

ALLAN SCOTT

(*From Union College, Schenectady, N. Y. and the Marine Biological Laboratory, Woods Hole, Mass.*)

INTRODUCTION

There is only one known example of paedogenesis in the Coleoptera and there are relatively few cases in the whole insect class. Hence any information which relates to the nature of paedogenesis in the beetle *Micromalthus debilis* has a general biological importance. One important question relative to paedogenesis in the beetle *Micromalthus* is: what is the mechanism which determines the strict separation of male production from female production in two types of larval mothers? This paper shows that the mechanism has an environmental rather than a genetic basis.

Many groups of animals produce unisexual broods. Thus some, aphids, Hymenoptera, Diptera, certain rotifers and Isopoda and some nematodes etc., produce broods of one sex and in some cases a partial explanation of the mechanism of this unisexual propagation is known. In many cases the broods are consistently female and involve a more or less constant process of diploid parthenogenesis, but in a few cases unisexual male progenies also occur. In the genus *Sciara,* Metz (1931) has disclosed a genetic basis which determines the sex of brood and a sex-linked gene is responsible. The thelytokous wasp, *Nemeritis canescens,* studied cytologically by Speicher (1937) is a perfect example of constant female production; no males were found in some fifty generations! The mechanism here also appears to have a genetic basis. It controls sex of progeny by determining a constant type of maturation. In the paedogenetic fly, *Miastor metraloas,* unisexual broods are apparently almost inviolably the rule and although Gabritschevsky (1928) ascribes this to a genetic mechanism, the contrary conclusion is indicated by Ulrich's (1936) work on *Oligarces.* Ulrich has shown that in the paedogenetic fly, *Oligarces paradoxus,* many broods show unisexual propagation although some broods contain both sexes. Hereditary differences among the larvae of *Oligarces* are not the determiners of the sex of the brood. It is the environment that is of primary importance.

It is the physiological state of the presumptive paedogenetic mother and the environment which primarily determine the sex of the brood in *Micromalthus,* just as in *Oligarces.* It is the purpose of this paper to describe a reversal of sex of brood which can be made to occur experimentally in the larval male-producer of *Micromalthus.* All the members of the first brood are male and all of the second brood are female.

Review of the Reproductive Types

In a previous paper (1938) I have described extensively the life history of *Micromalthus* and have outlined the reproductive anatomy of the various types. It is necessary for the purpose of discussion to review the reproductive types and more especially to describe the male producer with considerable care.

There are in the American variety of *Micromalthus debilis* five sexually mature reproductive types: (1) an adult female, (2) a female-producing paedogenetic larva, (3) a male-producing paedogenetic larva, (4) an adult male, and (5) a paedogenetic female larva with a mixed brood. This last is a modified male-producing larva and is the subject of research reported here. It is essential to note that the modified male-producer (amphoterotokous female) is simply a later developmental stage of the male producer. They rarely occur in nature but can be produced in large numbers experimentally. It is of incidental interest to refer here to the apparent absence of male-producers in the South African variety of *Micromalthus* which has recently been reported by Pringle (1938).

Normal History of the Male Producer

The male-producer (arrhenotokous female) is the only source of the adult male. She arises viviparously from a female-producing paedogenetic mother (thelytokous paedogenetic female) as one member of a large brood, sometimes twenty or more. In the first instar all these viviparous larvae possess legs which are lost at an early moult. They are all identical in appearance, indeed, it is impossible to distinguish the male-producer from other types until shortly before the last moult when inspection by dissection shows an ovary of a very special character in the male-producer. This early ovary is often recognizably distinct when only about 80 microns in length when a few egg cells (from one to five in each ovary) first begin to grow (Speicher, 1937). These continue to grow until they are of relatively large size and have become the shape of a hen's egg. The eggs of the thelytokous paedogenetic female are elongate so that the sex of the embryo resulting from either type

egg is predictable long before maturation. This adds another animal to the list showing sexual dimegaly of the ova (Wilson, 1925). When they are mature, the eggs of the male-producer begin development by haploid parthenogenesis in contrast to the diploid parthenogenetic development of the viviparous young (Scott, 1936). The one male that is successful in emerging from the mother is shed as a very young embryo in late June or early July. It is most peculiar, however, that although several embryos may be present in the ovary, only one is born. This new-born male remains for some four or five days adherent to the outside of the mother as is shown in Fig. 2 of Plate I. By that time he has developed sufficiently to insert his head into her genital aperture, which is shown at the arrow in Plate I, Fig. 1. Within a few days more the male has devoured his mother completely. These canabalistic males pupate and soon emerge as male adults.

This astonishing form of reproduction raises several perplexing questions. (1) Why is but one embryo shed by the male-producer when others equally advanced in development are present? (2) Is any one of the embryos more likely to be born than any other; viz., (*a*) does the position of the male in the mother have any bearing on successful emergence? Or (*b*) does the age of the embryo affect his ability to emerge? (3) Can any one of the other embryos be shed if the one that has been born is not allowed to feed upon the mother? (4) What becomes of the male-producer if her son is prevented from eating her?

THE BIRTH PROCESS

Why is only one male embryo shed by the male-producer? I can give no answer to the question but can only indicate some additional facts. Only seven male-producers in a group of three hundred and fifty-seven have given birth to two embryos. Fifty-eight male-producers have shed their male embryos in isolation. The females had previously been placed each in a shallow depression made in black wax and kept in a moist chamber. It is apparent from this that removal from their gallery in the wood does not affect their ability to give birth to the male embryo. Four of the fifty-eight individuals which shed in isolation,

---

PLATE I

Fig. 1. Feulgen preparation of male producer with one egg visible. The genital aperture is shown at the arrow.

Fig. 2. Male producer and successful male offspring.

Fig. 3. Ovary of the male producer with three embryos, all in the same developmental stage.

Fig. 4. Ovary of the reversing male producer with a newly-developed female embryo, and an exceptionally well-developed male embryo still within its follicle.
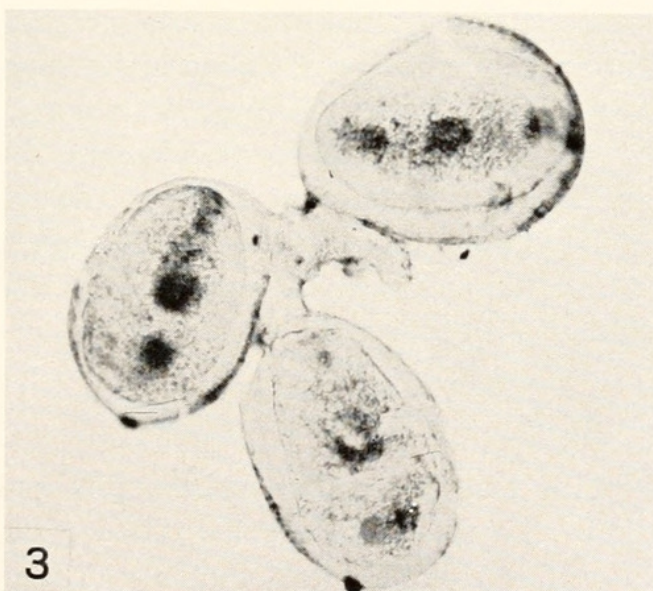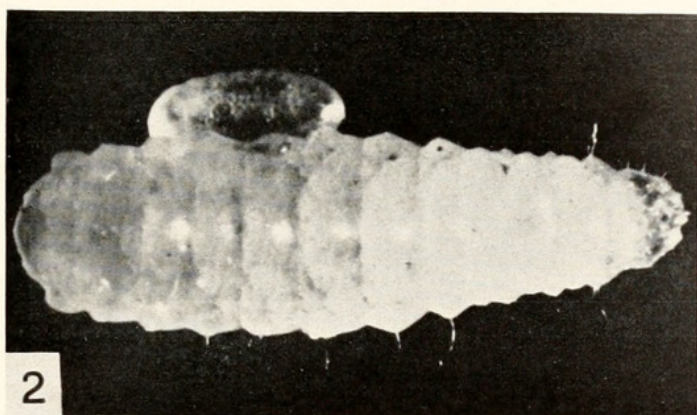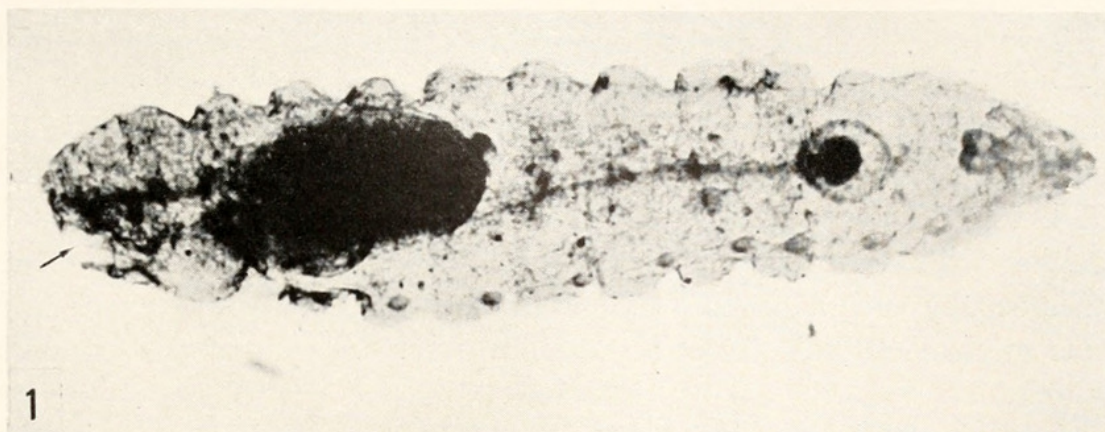
PLATE I

shed two eggs, thus it is possible that removal from the wood favors the birth of a second embryo.

Two other factors might conceivably affect the birth process, that is,—(1) position in the mother and (2) stage of development of embryo within the mother. I have previously shown that there is no favored position in the ovary from which an embryo is shed. (Scott, 1938, Fig. 13.) The successful male embryo may have occupied any position within the ovary. Indeed, the successful embryo may sometimes occupy such a position within the mother that it must experience some mechanical difficulty at birth, since other embryos appear to block its exit.

I do not think that the most mature male embryo is necessarily the most likely to emerge, for frequently the difference in age of the embryos is negligible, as is shown in Plate I, Fig. 3, and moreover, an embryo may occasionally develop into a rather well-developed larva while still within the follicle of the ovary, as illustrated in Plate I, Fig. 4.

No factual explanation of the mechanism governing this uniparity is available. However, a very plausible hypothesis can be formulated from the point of view of natural selection. The male has no other source of food during his larval life than his mother's body and since a second male would compete for this food supply, a process may have been developed which prevents this competition. This process very probably involves the active coöperation of the mother in that some internal physiological mechanism prevents a second male from being born. This mechanism is quite conceivably a failure of the muscular contractions which normally expel the egg. The continued presence of the born male on the mother is not necessary to prevent birth of the remaining embryos. The birth of one in some way sets the mother against further activity of the ovary and ducts. Does a hormone govern the contractions involved in the ovulation-birth process?

A New Brood in Male Producers

The fate of the mother after the emerged male has been removed is quite unexpected and is, I believe, a quite unprecedented observation. In practically every surviving case after approximately four weeks time a new, small brood is born. The members of the new broods have not yet been reared, nor have chromosome counts been possible, but the offspring are judged to be females with considerable certainty for the following reasons: (1) the shape of the egg is in every way similar to that of the female-producing female, (2) the development is in every way identical with that of the ordinarily produced females, and is vastly

different from the development of the male embryo, (3) the appearance of the newly-hatched embryos is identical with that of the more normally produced female larvae. That is, these second brood embryos possess well-developed legs and well-differentiated jaws, whereas new-born male

TABLE I

	Dead Dissected 7/24	Alive Dissected 7/25	Total	Percentage
Females showing female embryos on dissection	1	43	44	21.8
Females showing no female embryos	47	2	49	24.3
Females in which ovaries were not located	13	4	17	8.4
Died before examination			91	45.2

Total number involved in experiment—201. Mortality—75.7%

embryos are rarely beyond the germ band stage. The second brood larvae will, therefore, subsequently be referred to as females.

It should be stressed that this process is not an occasional one but is quite normal for those larval mothers that survive long enough. Thus in the summer of 1938, 93 females from which the male had been removed gave rise to a new female brood. Of this number, 21 mothers shed their brood and the rest showed female embryos on dissection. Since mortality records were not kept in 1938, the experiment was repeated in 1939.

On July 1, 1939, 201 females, each with the shed male removed, were isolated in black wax depressions in Syracuse dishes, 20 to each

TABLE II

New brood born before July 24 4
Found with female brood on dissection 7/24 10
Dead when dissected, ovaries disintegrated 8
Dead, no new brood developing 2
Lost 1
                                                          ——
        Total 25

dish and kept in a moist chamber. The mortality was severe, therefore the larvae were dissected before they could have given birth to their new brood. Table I summarizes the results.

In another experiment 25 male-producers were removed from the wood and the adherent male was removed from each one. They were likewise placed in a moist chamber at 35° C. ± 1°. The results are shown in Table II.

In this group 56 per cent developed a new brood of female embryos.

It is impossible from these data to decide whether or not every male-producer can, under favorable conditions, give rise to a new female brood but it is certainly indicated by the fact that out of the 63 animals that were still alive at the end of their respective experiments, only 6 did not show indications of a new brood. It is reasonable to expect that the larvae that died during the course of the experiment would also have given rise to a female brood had they survived.

The mortality, high in both experiments, is less severe at higher temperatures. The difficulty is largely due to the susceptibility of the larvae to mold. Perhaps a sterile technique would obviate the trouble.

Apparently no structural feature of the male producer prevents viviparity of the new female brood since a considerable number have been kept long enough to allow normal birth. The birth process is in every respect similar to that which takes place in the normal female-producing, paedogenetic female.

The size of the second brood of the reversing male-producer is intermediate between the size of normal female broods and the size of male broods. Normal female broods are frequently more than ten while male broods are rarely as many as four. An examination of the ovaries of forty reversing male-producers showed that the average number of new eggs formed was 4.2.

Study of the ovaries of this same group of forty reversing male-producers showed that a few females *failed to shed even one male, yet they developed a new brood of female larvae notwithstanding.*

The production of a new brood is not, therefore, absolutely dependent upon the experimental removal of the emerged male from his mother. Male-producers whose emerged male embryo dies will evidently give birth to a new all-female brood in the natural course of events. Indeed, in August, 1937, I found eighteen individuals with a new brood developing, obviously the result of this natural event. Dissection of these eighteen larval mothers showed an empty follicle from which a male had emerged and apparently died.

It seems altogether possible that a third brood might be produced by the original male-producer if it lived long enough. However, a single individual that lived thirty days after the production of the second brood showed no sign of new eggs when dissected.

It will be of some interest to test similarly the further reproductive capacity of the thelytokous paedogenetic female after the birth of her first brood.
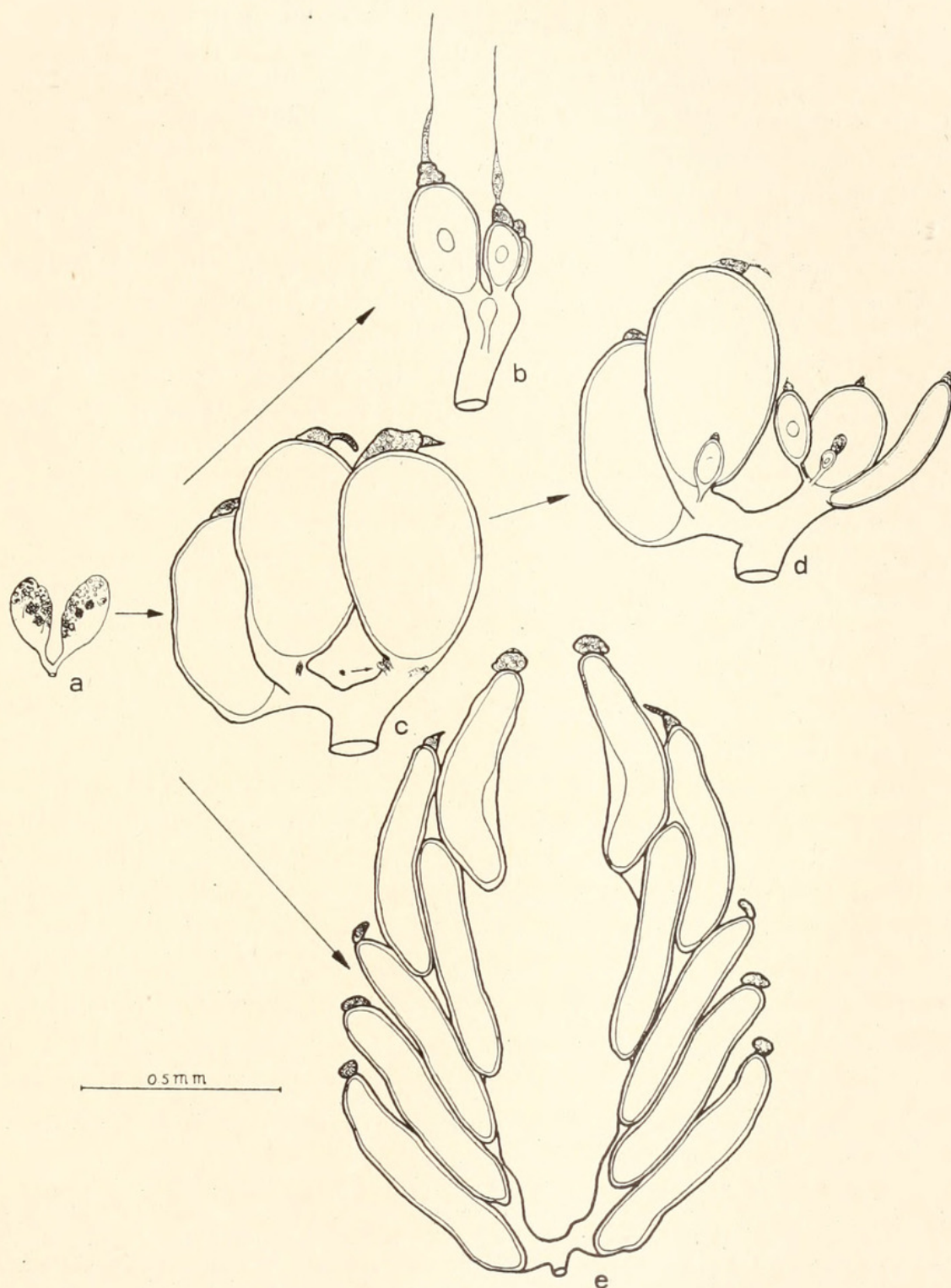
TEXT FIG. 1. A diagrammatic representation of the developmental possibilities of the basic ovary as it occurs in the several reproductive forms: (*a*) basic ovary (schematic) showing several undeveloped ovarioles in each ovary; (*b*) the ovary of the adult female with three ovarioles developing; (*c*) the ovary of the male producer (three ovarioles and eggs greatly enlarged, four others have remained small, see asterisk); (*d*) the ovary of the reversing male producer experimentally produced (the rudimentary ovarioles have enlarged and are developing female-producing eggs; (*e*) the ovary of the female producer with numerous ovarioles developing.

Histology of the Reversing Ovary

Studies of the ovaries of the male-producer indicate that the cells which give rise to the new crop of female-producing eggs are already present on the oviducts of the male-producer before the male embryo is born; indeed, they were probably present at the time of the first differentiation of the male-producing ovary. In the mature ovary of the male-producer these cells are located in little clusters around the ventral and lateral surfaces of the oviducts at the point of junction of oviduct and follicle. (Text Fig. 1c at the asterisk and Plate II, Fig. 5b). Structurally these groups of cells are undeveloped ovarioles. They doubtless represent ovarioles which did not enlarge during the first period of development of the male-producing eggs. The detailed structure of these ovarioles is shown in Plate II, Figs. 5 and 6. In both figures some differentiation can be seen within the ovariole and although no single egg cell can be identified with certainty, still, terminal cells of the germarium, nurse cells, and duct cells can be seen in Plate II, Figs. 6a, 6b, and 6c respectively.

In *Micromalthus* the ovaries of the four reproductive types are fundamentally similar. The general plan of the ovary in each of the female reproductive types is meroistic and acrotrophic, since the nutritive cells are all located at the apex of the ovariole. The variations in structure which the ovaries of the several reproductive types present may all be considered as modifications of a basic, undifferentiated ovary illustrated in Text Fig. 1a. This basic ovary possesses multiple ovarioles at the ends of a forked oviduct; it is the development or non-development of these potential ovarioles that determines the nature of the mature ovary. If the ovary develops within an adult female, then three or four of the ovarioles will enlarge with their contained eggs, as indicated in Text Fig. 1b. When, however, the basic ovary develops within a female-producing paedogenetic mother, a number of eggs, each in a different

---

PLATE II

Fig. 5. Frontal section of ovary of male producer: (a) Follicle; (b) undeveloped ovarioles (the follicle on the right side appears in another section); (c) oviduct; (d) last ganglion; (e) vagina.

Fig. 6. Oil immersion photograph of undeveloped ovarioles of the male-producing ovary. No enlargement has as yet taken place: (a) germarium; (b) potential nurse cells; (c) potential duct segment; (d) oviduct.

Fig. 7. Total Feulgen preparation of a reversing male-producing ovary: (a) unshed male egg; (b) female-producing egg; (c) oviduct; (d) new segment of oviduct; (e) empty follicle (out of focus); (f) vagina or terminal duct.

Fig. 8. Total Feulgen preparation of the ovary of a male producer with a new brood of female embryos: (a) female embryo; (b) empty follicle; (c) retained degenerating male embryo.
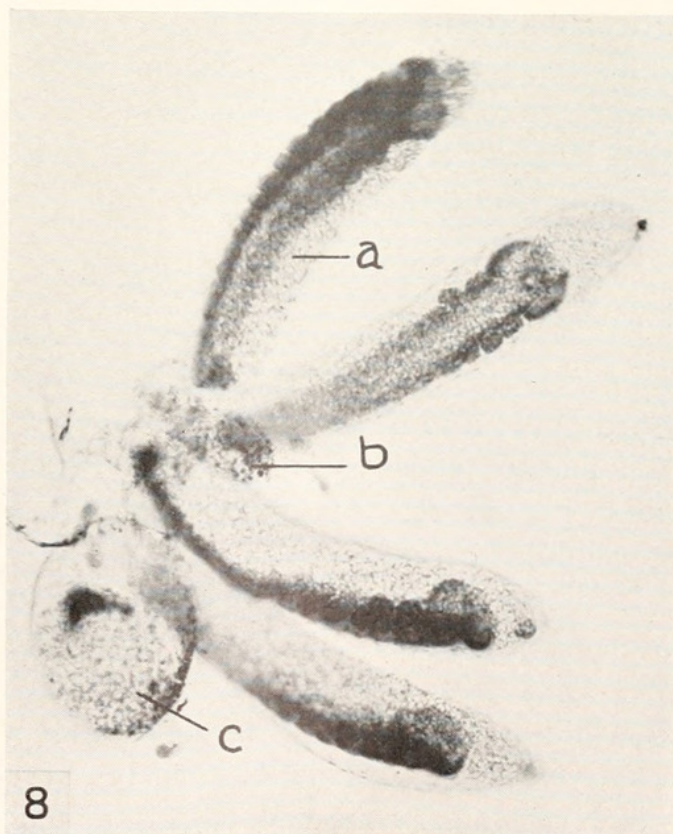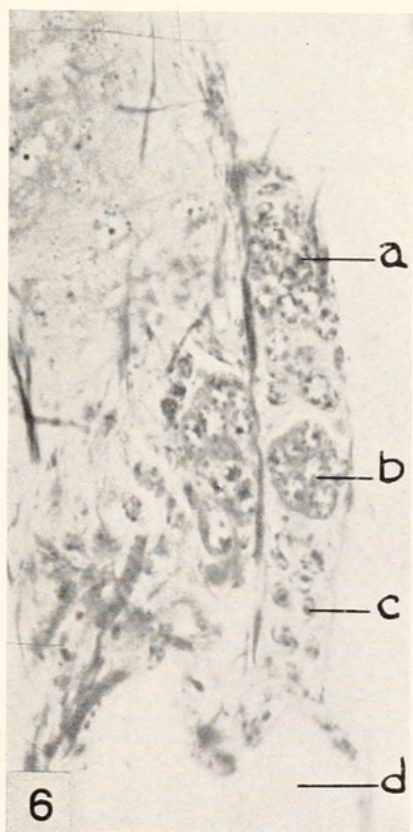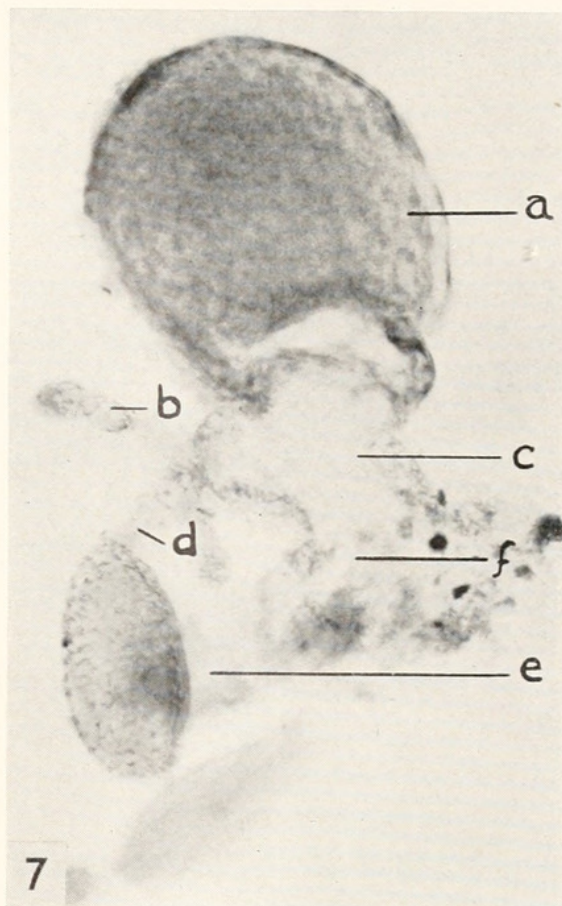
PLATE II

ovariole, enlarge to determine the characteristic ovary of that type of larva. This is illustrated in Text Fig. 1c. When the basic ovarioles develop within a male-producer, again only a few of the ovarioles enlarge. In this case, too, only a few eggs develop, one in each ovariole, so that the fully developed ovary of the male-producer, shown in Text Fig. 1c, still possesses a number of undeveloped ovarioles at the base of the enlarged follicles. It is these undeveloped ovarioles that enlarge to give rise to the second all-female brood under the conditions noted above and illustrated in Text Fig. 1d.

The development of the new eggs involves the production of other new parts of the reproductive system, for although the old oviducts and vagina are utilized by the larvae of the second brood on emergence, it will be apparent from Plate II, Figs. 6c and 7d, that a new segment of oviduct is added during the development of the new crop of eggs. The potentialities of the ovariole tissue are such, therefore, that it gives rise to the following reproductive structures: (a) oviduct, (b) follicle cells, (c) eggs, one per ovariole, (d) nurse cells and (e) germarium. The new oviduct segment is at first relatively long, but it is inconspicuous in late development, as Plate II, Fig. 8 shows. Perhaps it is incorporated into the follicle as the egg grows.

It should be added that the development of the new eggs is not particularly related to the stage of development of the retained males, for the latter may be in any stage of development from a post-maturational stage to a well-developed larva. Frequently, indeed, the retained males undergo an abnormal type of development which also has no apparent effect on the new brood.

SUMMARY

1. The paedogenetic, arrhenotkous female in the beetle, *Micromalthus debilis* (Leconte), gives birth to but one male embryo, although unshed males also develop.

2. Factors which determine this uniparity are still uncertain, but neither greater age nor more favorable position in the mother are determining factors.

3. When the single successful male offspring is not allowed to devour his mother, a new crop of eggs develops in the ovary.

4. These new eggs are all of the elongate female type. They develop into a larva identical in appearance with the first stage larva of the thelytokous paedogenetic female.
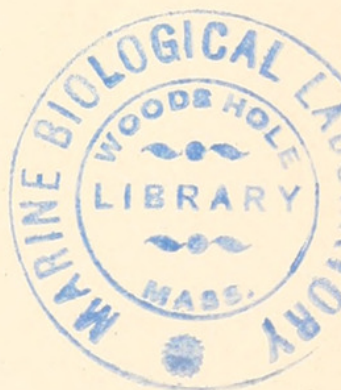
5. Histologically the new eggs originate from undeveloped ovarioles which failed to develop during the first period of growth of the male-producing eggs.

6. Sex of brood in *Micromalthus* is obviously determined by environment, intrinsic or extrinsic, and not by the hereditary constitution of the mother.

BIBLIOGRAPHY

GABRITSCHEVSKY, E., 1928. *Bull. Biol. de la France et Belg.,* vol. **62**.

METZ, C. W., 1931. *Genetics,* vol. **16**.

PRINGLE, J. A., 1938. *Trans. Roy. Ent. Soc.,* vol. **87**.

SCOTT, A. C., 1936. *Jour. Morph.,* vol. **59**.

SCOTT, A. C., 1938. *Zeitschr. für Morph. und Ökol.,* Bd. **33**.

SPEICHER, B. R., 1937. *Jour. Morph.,* vol. **61**.

ULRICH, HANS, 1936. *Zeitschr. für ind. Abst. und Vererb.,* Bd. **71**.

WILSON, E. B., 1925. The Cell in Development and Heredity. Third Edition.
     The Macmillan Co., New York.