

Composite eggs and Siamese twins in paedogenetic gall midges

By DIRK F. WENT¹ AND RENÉ CAMENZIND²

*From the Department of Zoology, Swiss Federal Institute of
Technology, Zurich*

SUMMARY

Several types of composite eggs were found in two species of paedogenetic gall midges. One composite egg, consisting of two blastoderms, was cultured *in vitro*, and its development was recorded on film. Two other composite eggs were fixed in the oocyte stage; each contained two nurse chambers, two egg chambers and one oocyte nucleus. Some other 'eggs' are described, which consisted merely of a nurse chamber or of one or two oocytes and of the follicular epithelium. It is argued that the formation of composite eggs and incomplete eggs must be due to errors in the formation of the oocyte-nurse cell complexes.

The article also describes three Siamese twins in larval stage which were found in two paedogenetic gall-midge species. In two twins the larvae were linked posterior–posterior, while in the third they were connected anterior–anterior. It is assumed that the Siamese twin-larvae evolved from composite eggs.

INTRODUCTION

Among insects composite eggs are particularly frequent in phasmids (Pijnacker & Diephuis, 1970), where they consist of two or more oocytes surrounded by the follicular epithelium and arise by fusion of oocytes in the ovary.

In gall midges Counce (1968) was the first to notice composite eggs. In this insect family some species can reproduce parthenogenetically in the larval stage (= paedogenesis; see Ulrich, 1962). The eggs develop through embryogenesis in the haemolymph of the mother larva and grow considerably (Ivanova-Kasas, 1965). By examining eggs of the paedogenetic genus *Miastor*, Counce detected among thousands of normal eggs 14 composite eggs, all composed of two oocytes or embryos respectively. In *Heteropeza pygmaea*, another paedogenetic gall midge and a close relative of *Miastor*, she did not find any composite eggs, nor have they been mentioned by any other investigator of *Heteropeza*.

In the present article we can report the observation of a composite egg in the

¹ *Author's address:* Zoologisches Institut (I), Universität Würzburg, Röntgenring 10, 87 Würzburg, Germany.

² *Author's address:* Department of Zoology, Swiss Federal Institute of Technology Zurich, Universitätsstr. 2, 8006 Zürich, Switzerland.

blastoderm stage of *Heteropeza*. This egg was found in an *in vitro* culture of *Heteropeza* eggs (Went, 1971) and its development *in vitro* was recorded on 16 mm film. In *Heteropeza pygmaea* and in another paedogenetic species, *Mycophila speyeri* (for life-cycle see Ulrich, Petalas & Camenzind, 1972), we found a new type of compound egg to which we also would like to apply the expression 'composite egg', since the mode of origin probably is the same.

In *Miastor* no development of composite eggs beyond the stage of germ band extension has been observed (Counce, 1968). Also, in *Heteropeza*, no composite eggs in advanced developmental stages have been found as yet. However, two Siamese twins of *Heteropeza* larvae and one of *Miastor* larvae were discovered, which possibly had developed from such composite eggs. In the present article composite eggs and Siamese twin-larvae are described; the mode of formation of composite eggs and their possible development to Siamese twin-larvae are discussed.

MATERIALS AND METHODS

Our investigations were carried out with three paedogenetic species – *Mycophila speyeri* (Ulrich, Petalas & Camenzind, 1972), *Heteropeza pygmaea*, line 2K (Camenzind, 1966) and *Miastor metraloas* – all members of the gall-midge family (Itonididae, syn. Cecidomyiidae; Diptera).

Details of the culture method for female-mothers, i.e. mother larvae which produce only female eggs, are given by Camenzind (1962) and Went (1971). The culture method for male-mothers and male-female-mothers, i.e. mother larvae which produce only male eggs or both male and female eggs, is described by Hauschteck (1962) and Camenzind (1962). The cultures were kept at 25 °C.

The composite eggs in the oocyte stage were stained with orcein-lactic acid (Camenzind, 1966).

The composite egg in the blastoderm stage was cultured *in vitro* at 25 °C according to the method of Went (1971) and filmed with a 16 mm camera, inverted microscope and time-lapse equipment (details in Went, 1972). The exposure frequency was 1 frame/20 sec.

DESCRIPTION OF EGGS AND LARVAE

In this paper the term 'egg' refers to the total content of the follicular epithelium. In normal development this consists of the oocyte and the nurse syncytium, during oogenesis, or the embryo in subsequent stages where, in the absence of a chorion, the follicular epithelium functions as the egg membrane. The term 'egg chamber' refers to the oocyte (with or without nucleus), the term 'nurse chamber' to the nurse syncytium; both chambers are located within the follicle.

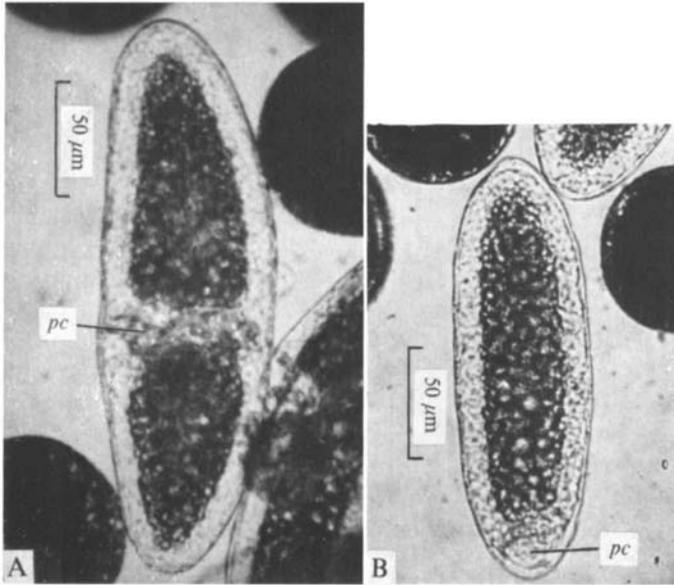


Fig. 1. Composite egg (A) and normal egg (B, from another preparation), both in the blastoderm stage at the 12th cleavage division. The posterior ends of both blastoderms in (A) lie in the centre of the egg. The posterior pole of the egg in (B) is the lower pole in the figure. *pc* indicates the location of the pole cells. Both pictures are magnifications from 16 mm film.

Composite egg in the blastoderm stage

Among more than 20000 female eggs examined to study embryogenesis of *Heteropeza pygmaea*, we found only one composite egg. It was discovered after tearing apart a young female-mother in order to start an *in vitro* culture with her eggs. These eggs, the composite egg included, were in the blastoderm stage. The composite egg was easy to distinguish from the other eggs because of its unusual form and large size (Fig. 1). The posterior ends of the two components were in the middle of the egg. Thus, the orientation, according to the classification of Counce (1968), was posterior–posterior. The number of pole cells lying between the blastoderms could not be determined, but there seemed to be two areas with pole cells, one belonging to either blastoderm.

We were able to film the composite egg over a period of 14 h until it degenerated. In Fig. 2 we have recorded some data taken from the time-lapse film. In both blastoderms of the composite egg the yolk globules undergo alternative periods of oscillation and rest within the yolk syncytium. From a comparison with the data of Went (1972) we conclude that during the two resting periods (in either blastoderm) the 12th and 13th cleavage divisions take place. The upper blastoderm in Fig. 1A starts the 12th cleavage division 1 h ahead of the lower one. It is about 25 μm longer than the lower blastoderm and this difference in size persists during degeneration of the composite egg (see below).

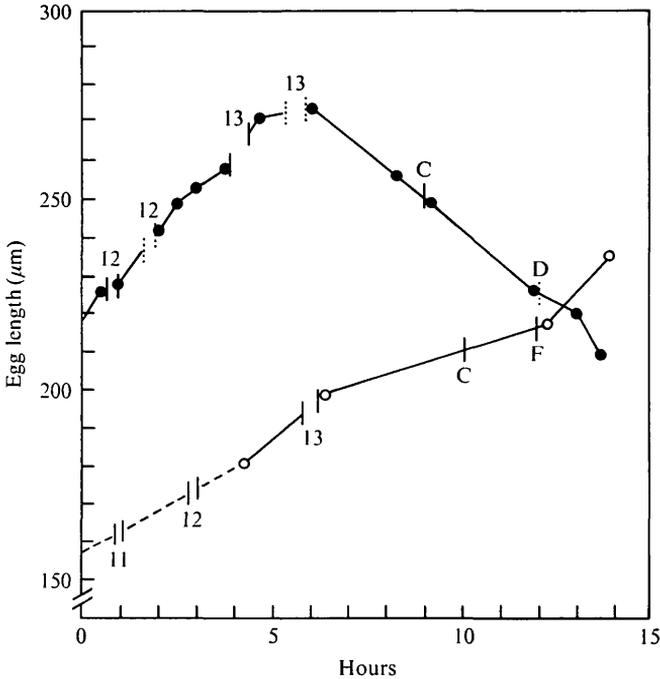


Fig. 2. Length curves and alternation of oscillation and rest of the yolk globules in the composite egg (●—●) and in a normal blastoderm from the same culture drop (○—○). Zero time on the abscissa marks the beginning of the filming. For the composite egg the periods with resting yolk globules, corresponding to the 12th and 13th cleavage divisions, are indicated by vertical continuous lines (upper blastoderm in Fig. 1 A) and dotted lines (lower blastoderm in Fig. 1 A) respectively. D, The first signs of degeneration in the composite egg; C, cessation of oscillation of the yolk globules in both eggs. The length of the normal egg could not be measured up to the 4th hour; the line of dashes is extrapolated. At F formation of the germ band begins.

Three hours after the 13th cleavage division of the lower blastoderm the oscillation of the yolk globules gradually ceases in both blastoderms; in normal *in vitro* cultured eggs this occurs about 4 h after the 13th cleavage division and 2 h before germ-band formation. The decrease in length (implying a decrease in size) of the composite egg precedes the visible onset of degeneration, as it does in 'normal' degenerating eggs (Went, 1972). This degeneration starts in the lower blastoderm at about the 12th h after the beginning of filming with disorganization of the yolk syncytium. Formation of the germ band, which in growing eggs in the *in vitro* culture takes place about 6 h after the last cleavage division, did not occur in the composite egg.

Some data for a normal blastoderm from the same preparation also are recorded in Fig. 2 for comparison. The rate of development (duration of cell cycles) of the composite egg is lower than that of the normal egg, which is unusual for eggs within one culture (Went, 1972).

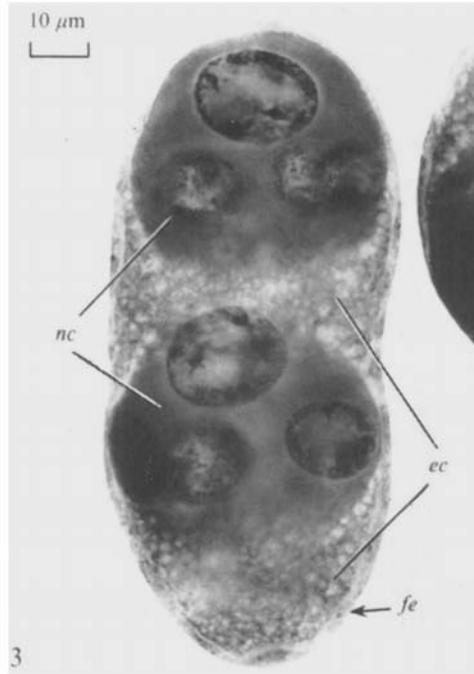


Fig. 3. Composite female egg in *Mycophila speyeri*. *nc*, Nurse chamber; *ec*, egg chamber; *fe*, follicular epithelium.

Composite female egg in Mycophila speyeri

This egg was found in a microscopic preparation which otherwise contained only female eggs taken from young female-mothers. It consists of two nurse chambers of normal size and two egg chambers, one of which lies between the two nurse chambers while the other is at the lower end of the egg in Fig. 3 and has a normal crescent-like shape. The egg is surrounded by the follicular epithelium.

In normal eggs of the same age, i.e. at the beginning of the growth phase, the oocyte nucleus becomes invisible; only a spherical region in which yolk globules are absent can be seen with the Nomarski interference microscope. A careful search in our composite egg revealed only one such region in the lower egg chamber.

Composite male egg in Heteropeza pygmaea

This egg was discovered in a microscopic preparation which contained eggs from male-mothers and male-female-mothers. Fig. 4 shows two micrographs taken at two different levels. The egg consists of two nurse chambers and two egg chambers. The upper one is quite small whereas the lower one has grown to a size found also in normal male eggs in the same developmental stage. In the upper egg chamber two pairs of so-called small nuclei can be seen, i.e. nuclei

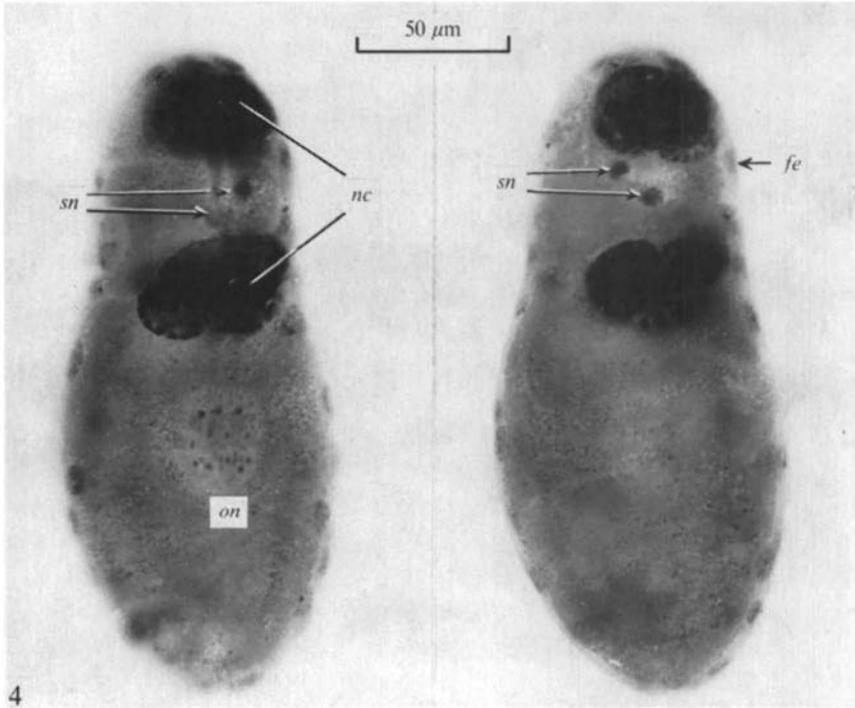


Fig. 4. Composite male egg in *Heteropeza pygmaea*, photographed at two different optical levels. *nc*, Nurse chamber; *on*, oocyte nucleus; *fe*, follicular epithelium; *sn*, small nuclei.

which are of maternal origin and regularly occur in male eggs. They later fuse with the egg nucleus after two meiotic divisions have reduced its chromosome number (for details see Hauschteck, 1962; Camenzind, 1971). The lower egg chamber contains no small nuclei, but an oocyte nucleus which is in prophase of the first meiotic division. The whole egg is surrounded by the follicular epithelium.

Incomplete eggs in Heteropeza pygmaea

In microscopic preparations with eggs (Fig. 5A) and embryos from female-mothers we have found incomplete eggs which consist only of a nurse chamber (Fig. 5B) or of one or two oocytes (Fig. 5C). They are surrounded by the follicular epithelium. The incomplete eggs are more frequent than composite eggs. In 8 preparations with a total number of about 1200 eggs and embryos we found 25 eggs with a nurse chamber only and 5 eggs with one or two oocytes only.

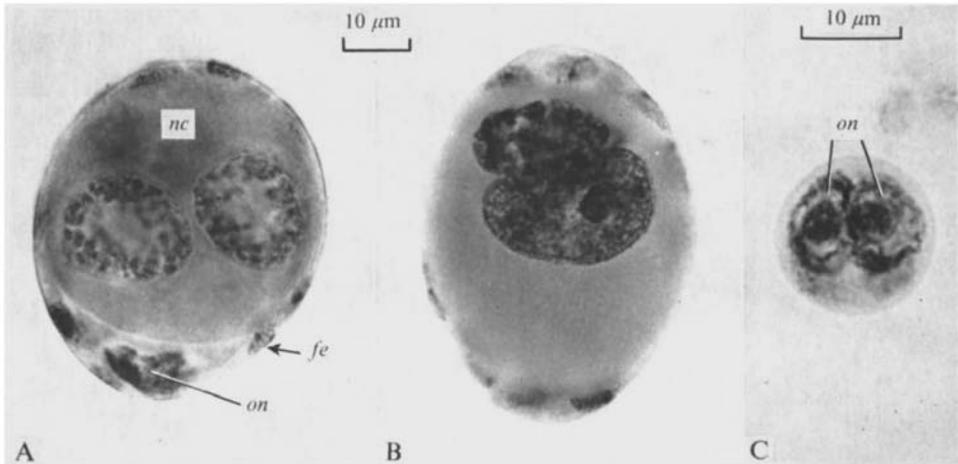


Fig. 5. *Heteropeza pygmaea*. (A) Normal female egg. (B) Incomplete egg consisting of a nurse chamber only. (C) Incomplete egg consisting of two oocytes only. Both incomplete eggs are surrounded by the follicular epithelium. *nc*, Nurse chamber; *on*, oocyte nucleus; *fe*, follicular epithelium.

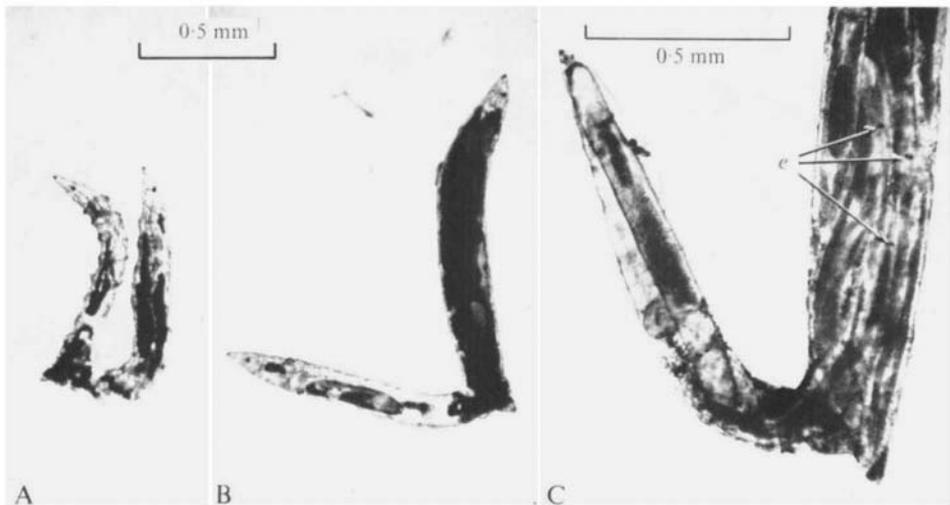


Fig. 6. A pair of Siamese twin-larvae in *Heteropeza pygmaea* at different times after hatching. For details see text. *e*, Eyes.

Siamese twins in Heteropeza pygmaea

One pair of twins was found in a culture dish in which all the other female larvae reproduced paedogenetically and became female-mothers. The twin-larvae were discovered about 1 day after they had hatched from their mother (Fig. 6A). The two larvae are connected at their posterior ends. The left larva is slightly shorter and its fat body smaller than that of the right larva whose size and fat body correspond to those of a normal larva of the same age.

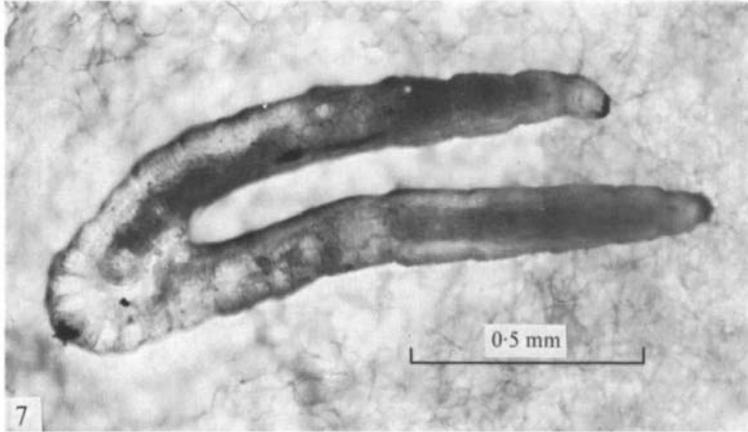


Fig. 7. A pair of Siamese twin-larvae in *Miastor metraloas*.

In Fig. 6B the twin-larvae are about 3 days old. The right larva contains seven embryos whereas the left larva contains only one embryo which is in the stage of the contracted germ band. One day later (Fig. 6C) dorsal closure is nearly finished in the embryos. In some of them the eyes can be seen. The embryos at the posterior end of the right mother larva sometimes moved into the left mother larva. This shows that at least at the end of paedogenetic development the body cavities of the twins were connected. When the twins were 5–6 days old, eight larvae hatched out. They were transferred to a fresh culture. Seven larvae developed into normal female-mothers while one larva died.

Another pair of Siamese twin-larvae was discovered a few years earlier. In these twins, also, the posterior ends were connected, but ventro-laterally. Both larvae were equal in size and somewhat smaller than normal larvae of the same age. Since they tried to crawl in opposite directions, they were handicapped in their locomotion, and hence in feeding. Both larvae produced several daughter larvae.

Siamese twins in Miastor metraloas (Fig. 7)

These Siamese twin-larvae were found about 3 days after they had hatched from their mother in a culture which otherwise contained only female larvae. The twin-larvae were connected back to back at their anterior ends; they had two pairs of antennae and two pairs of eyes. They did not feed, possibly because neither larva was able to lie on its ventral side. The larvae died when they were about 8 days old. They were then fixed and sectioned. Although the inner organs had already begun to deteriorate it could clearly be seen that the two larvae had a common haemocoel and that both larvae had moulted and become hemipupae. There was some indication that the digestive tracts of the two larvae were connected with each other and possibly not with the mouth parts, which would have been the main reason that the larvae were not able to feed.

DISCUSSION

In paedogenetic gall midges, as in other gall midges, organized egg tubes with a germarium are absent (Kahle, 1908; Ivanova-Kasas, 1965; Counce, 1968; Matuszewski, 1968). The ovary of *Heteropeza* contains oogonia, all developing into oocytes, and cells of mesodermal origin, developing into nurse cells or into follicle cells (Panelius, 1968). The number of trophocytes constituting the syncytial nurse chamber is variable (Reitberger, 1940; Panelius, 1968). At the time of egg follicle formation in the ovary a number of small mesodermal cells forms a layer – the follicular epithelium – around an oocyte and its associated cluster of nurse cells. A chorion is not formed and the follicular epithelium functions as egg membrane till the end of embryogenesis.

In the paedogenetic gall midges different abnormal egg types have been observed (Counce, 1968; this article). These egg follicles are all enclosed by the follicular epithelium and consist in the case of composite eggs of two (sometimes perhaps one) nurse chambers and two egg chambers with one or two oocyte nuclei, in the case of incomplete eggs merely of a nurse chamber or of one or two oocytes.

According to Counce (1968), who described only composite eggs but no incomplete eggs, composite eggs in *Miastor* might be formed in two ways: by the failure of follicle cells to ingrow and separate two oocyte-nurse cell complexes or by the association of two oocytes with one group of nurse cells. Our findings of both composite and incomplete eggs indicate that the cells forming the follicular epithelium do not control the formation of the egg follicle from the proper components, i.e. one oocyte plus one nurse chamber. By the time the mesodermal cells transform into follicle cells, the oocyte-nurse cell complexes have been established (Kahle, 1908). The follicle cells presumably form a layer around any associated complex of oocytes and nurse chambers or any single oocyte or nurse chamber, which at that time is present in the ovary. Thus the faulty composition of composite eggs or the formation of incomplete eggs seems to be due to errors in the formation of the oocyte-nurse cell complexes.

Composite eggs of paedogenetic gall midges in stages of embryonic development beyond germ-band extension have been observed neither by Counce (1968) nor by us. Failure to detect composite eggs in advanced developmental stages is probably the result of premature degeneration, such as was observed *in vitro* for the composite egg in the blastoderm stage. Degeneration of eggs and embryos is indeed a common event *in vivo* as well as in the *in vitro* cultures. The percentage of degenerating eggs and embryos in well-fed larvae is at least 30 % on an average and rises up to more than 95 % in undernourished larvae. In the *in vitro* cultures on an average more than 65 % of the embryos degenerate and dissolve (Went, 1971, 1972). Therefore we presume that degeneration of the composite eggs during embryonic development is the consequence of malnutrition and not of some incompatibility of the two partners of a composite egg.

Furthermore, our discoveries of Siamese twin-larvae seem to demonstrate that at least some composite eggs developed through the whole of embryogenesis. However, since the Siamese twins were detected only after their hatching from the mother larvae, we have no proof that the twins actually developed from composite eggs.

Theoretically, Siamese twins can evolve either by partial splitting of the egg in early embryogenesis (= incomplete polyembryony) or by fusion of two eggs or embryos. It is highly improbable that the twins which we described were the product of incomplete polyembryony, since normal polyembryony is very rare and has never been observed in gall midges. It seems more reasonable to assume that our Siamese twin-larvae had their origin in a fusion of oocytes or of embryos. A fusion of two embryos which are drifting independently in the circulating haemolymph is not very likely to occur. Therefore the most probable explanation for the origin of Siamese twin-larvae is the fusion and development of two oocytes within the same follicular epithelium.

Thus, the mode of origin of the Siamese twin-larvae is in all likelihood completely different from that of Siamese twins in the traditional sense of the word, which result from partial splitting of the egg in early embryonic development.

The authors would like to express their thanks to Dr M. Madhavan and Mrs M. Braun for their skilled technical assistance.

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(Manuscript received 17 April 1972, revised 17 May 1972)

