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Mode and Timing of Body Pattern Formation (Regionalization) in the Early Embryonic Development of Cyclorrhaphic Dipterans (*Protophormia, Drosophila*)*

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Summary. 1. Eggs of the blowfly *Protophormia* spec. were separated into anterior and posterior fragments of varying sizes. The operations were carried out between oviposition and the blastoderm stage. The partial larvae produced by the fragments were scored for the cuticular pattern they had formed.

2. The cuticle of the 1st instar larva carries 11 denticle belts which correspond to the anterior borders of the thoracic and abdominal body segments. These are considered the elements of a linear longitudinal pattern which starts with the head region.

3. Egg fragments of the sizes studied did not produce the complete cuticular pattern.

4. If denticle belts were present on the partial larvae formed in egg fragments, these always included the corresponding terminal pattern element (no. 1 in anterior, no. 11 in posterior fragments). Bigger partial patterns from anterior fragments may have any belt up to no. 10 as their most posterior belt, posterior partial patterns may start anteriorly with any belt up to no. 1, i.e. behind the head region.

5. After fragmentation during early stages of development, all eggs fail to form some pattern elements. Fragmentation thus causes a gap in the pattern. Extent and position within the pattern of this gap depend on level and stage of fragmentation.

6. With increasing egg age (developmental stage) at fragmentation, the gap in the cuticular pattern becomes progressively smaller. Eggs fragmented during or after formation of the blastodermal cell walls as a rule form all pattern elements.

7. The progressive reduction of the gap in the cuticular pattern is due to formation of bigger sets of pattern elements in *both* partner fragments. I.e. on the average an anterior or posterior fragment of given size will produce more pattern elements if separated from the rest of the egg at a later stage than if separated early.

8. In order to produce a given set of pattern elements, a fragment needs to be bigger on the average when separated early than when separated later on. This applies to both anterior *and* posterior fragments of the fragmentation levels studied.

9. According to these results, the egg of *Protophormia* cannot be considered a mosaic of determinants for the different pattern elements at oviposition. The developmental fate of at least the more equatorial egg regions appears to become specified epigenetically during the period between oviposition and blastoderm formation.

10. Once the egg has become subdivided into blastoderm cells, it reacts as a developmental mosaic with respect to the pattern studied.

11. Preliminary results in *Drosophila* are compatible with these conclusions.

12. The results are compared to those obtained from other insect groups, and formal models for their interpretation are discussed. Pattern specification by interaction of terminal egg regions can be considered the common denominator for a number of egg types.

13. The results demonstrate that formally comparable processes of pattern formation occur in different insect egg types at different stages of development.

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Introduction

The embryogenesis of higher dipterans (Cyclorrhapha), although well described (see Anderson, 1966) and frequently analyzed with the aid of gene mutations (see Wright, 1970), has only rarely been subject to the methods of classical experimental embryology, and with comparatively little success (see Counce, 1961; Krause and Sander, 1962). Fragmentation of the developing system, although apparently somewhat outdated, more than any other method excepting transplantation permits conclusions concerning the spatial distribution of various functional components of the system at different times. Such conclusions are necessary for the understanding of some fundamental aspects of embryogenesis, especially the processes involved in regionalization (Waddington, 1966), i.e., the specification of its future course of development to each egg region, also referred to as body pattern formation. Information on the functional topography of the developing system should be particularly interesting in species which, like *Drosophila*, are accessible to the methods of genetics and molecular biology. Some 20 years ago, Poulson (1950) discussing the "ground plan" of the embryo stated that in *Drosophila* "a really thoroughgoing experimental analysis remains to be done". With respect to determination of the basic body pattern, this holds true still to-day. Two reasons may chiefly be responsible for this lack of information: the ill-founded, but steadily perpetuated notion of the dipteran, and particularly, cyclorrhaphan, egg as being a strictly "mosaic type" egg (e.g. Schleip, 1929; Anderson, 1966), and the fact that cyclorrhaphan eggs as compared to other insect eggs prove rather intractable with the techniques of classical experimental embryology. The former fact discouraged experimental analysis of pattern forming processes because these ought to occur during oogenesis and would therefore be inaccessible to the usual experimental treatment, and the latter fact prevented accumulation of a more or less coherent body of data. Recently, some technical obstacles, as far as preparation and rearing of egg fragments are concerned, have been reduced by a novel technique (see Sander, 1971). This technique was applied to the cyclorrhaphic dipterans *Protophormia* spec. and *Drosophila melanogaster*. Owing to its smaller size and greater lability, the egg of *Drosophila* was abandoned after some initial results had been obtained; since then the technique has been improved and further studies in *Drosophila* will follow. Meanwhile, a bulk of data was collected on *Protophormia* and this forms the main part of the present paper. As all authors agree that embryonic development in the higher dipterans is remarkably uniform (e.g. Anderson, 1966), it may be expected that conclusions drawn from experiments on *Protophormia* eggs may hold, with some minor modifications, for *Drosophila* as well.

I. Experiments on *Protophormia* Eggs

Material and Methods

The eggs investigated came from a stock of *Protophormia* spec. (probably P. terrae novae) originating from specimens caught at Freiburg i. Br. The average length of the eggs is 1.2 mm and the average maximum diameter 0.28 mm. The egg is of ovoid shape and tapers somewhat more towards the anterior pole which thus can be identified. The ventral egg side is convex, the dorsal side flat or slightly concave. The eggs were deposited by the flies on meat cubes offered in a highly humid atmosphere at 28–32° C. As checking for eggs disturbs the flies and keeps them from egg laying, the meat cubes were normally inspected only at 15 min intervals,

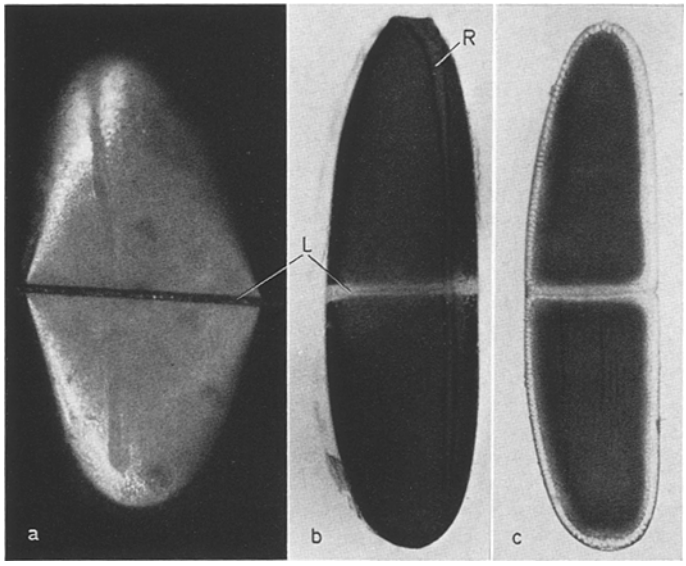


Fig. 1a—c. Early effects of fragmentation on the egg of *Protophormia* spec. a) Egg separated into 2 fragments, seen through the supporting slide of the fragmentation device. The eggs were kept in this position until larval cuticle had been formed. Magn. ca. ×70. b) Egg after removal from the fragmentation device, photographed in transmitted light. At the fragmentation level, the structure of the chorion has been changed so as to become translucent. Magn. ca. ×60. c) Egg removed from the device after blastoderm formation, dechorionated. Note that both fragments have formed a continuous layer of blastoderm cells at the plane of fragmentation. Magn. ca. ×60. *L* level of fragmentation, *R* rhaphe in the dorsal midline of the chorion

which occasionally were reduced to 10 min or extended to 30 min according to strength of oviposition tendency in the females. To avoid experimenting on eggs which had started development before oviposition, flies were allowed to oviposit under optimum conditions for some time before the meat for the experimental eggs was introduced into the cage. The eggs collected were kept at 22° C until they had reached the stage required for fragmentation.

The eggs of a cluster were separated by treatment in 5% Na_2S (5 min) followed by several rinsings. They may then be dechorionated in a solution containing sodium hypochlorite, but this often interferes with further development, especially in young eggs. Therefore, the eggs used for fragmentation were not dechorionated. Their stage at fragmentation was estimated by time since oviposition, and then ascertained by "double checking". For this purpose the whole egg batch was divided into 3 lots. Only one of these was used for fragmentation and therefore not dechorionated. Of the remaining two lots, one was dechorionated with sodium hypochlorite and observed shortly before the experimental eggs were fragmented, and the other immediately after the eggs had been fragmented. If both control lots turned out to be homogeneous in appearance as well as in stage and rate of development, the fragmented eggs were considered of normal developmental capacity and assigned to the stage of development revealed by the control lots.

For fragmentation of the eggs the device of Sander (1971) was used. It separates the contents of the egg into 2 fragments without severing the vitelline membrane. This is achieved by pressing the eggs with the blunted edge of a razor blade against a transparent plastic foil supported by a glass slide. At the end of the separation procedure which was adjusted to take about 2 min, the edge of the blade is separated from the foil only by 2 layers of vitelline membrane, whereas the egg cell or egg plasmodium has been separated completely into 2 frag-

ments (Fig. 1a). The separation is quite tight, as can be demonstrated by puncturing one of the fragments. The eggs, normally 10, sometimes 20 at a time, were before fragmentation lined up along a mark indented into the plastic foil by a previous lowering of the blade. They were left in the apparatus and kept in water at 25° C. On having reached a stage of development corresponding to the hatching stage in normal development, the eggs were released from the apparatus. The position of the mark left by the blade on the chorion (Fig. 1b) was noted in order to calculate the fragment sizes (see below). The eggs were then singly dechorionated and transferred into a drop of glycerine on a slide. A cover glass was applied and pressed slightly until the vitelline membrane burst. This treatment flattens out the partial larvae formed in the fragments and thereby enables the structures formed to be observed under high magnification. The slides with the glycerine-embedded cuticles were stored for reference.

Fragment size (fragment length) is correlated to the mark left by the blade in the chorion. The distance of this mark from the posterior pole was measured and expressed as per cent of the length of the whole egg (% EL). This value, called the level of fragmentation, is representative for the length of both fragments; with increasing % EL, the posterior fragment becomes longer, and the anterior fragment shorter. However, the correlation between the mark in the chorion and the actual plane along which the egg's contents are separated is not quite linear. Mark and plane coincide if the separation is carried out near the middle of the egg (50% EL). They do not coincide if the blade separates the egg more terminally. In this case the plane of fragmentation of the egg's contents is situated closer to the pole than the mark in the chorion. In a test series, chorion marks situated around 30% EL or 70% EL were found to be correlated with fragmentation planes situated closer to the corresponding egg pole by ca. 5% of the egg's length (see Fig. 21 in Herth, 1970). This discrepancy which was noted earlier in other insect eggs (Sander, 1959) is due to the volume/surface ratio of the fragments which decreases with fragment size. This forces some material from the smaller into the bigger fragment before the separation is complete. If separation is carried out slowly as indicated above, the shifting of material occurs in quite an orderly manner and does not lead to any discruptions or turbulences (see Fig. 1c). The shifting process is essentially similar in all stages operated upon and therefore cannot account for the differences in pattern forming ability of fragments from different stages as revealed in this study.

The geometry of the egg limits the possible sites for fragmentation to the area between roughly 20% and 70% EL; if the blade is applied more terminally the eggs slide away instead of being fragmented.

Normal Development and Stages Selected for Fragmentation

The early embryonic development of *Protophormia* spec. does not deviate significantly from the early development of other cyclorrhaphic dipterans (compare e.g. Fish, 1947; Sonnenblick, 1950; Formigoni, 1954; Schoeller, 1964). The later embryonic development corresponds essentially to the descriptions given by Breuning (1957) and Schoeller (1964). In fragmented eggs, the complicated movements leading to the involution of the head may be repressed to some degree. Apart from this, there are no observations indicating that the course of development of the structures eventually formed is different in fragments as compared to intact eggs.

The rate of development at 22° C varies somewhat in individual eggs. On the whole it corresponds closely to that observed by Formigoni (1954) and Nitschmann (1961, see Krause and Sander, 1962). Segregation of pole cells occurs between 105 and 120 min after oviposition. The cellular blastoderm stage is reached between 170 and 180 min after oviposition, and the head folds form between 190 and 210 min. In fragmented eggs, the development up to the hatching stage is retarded by a few hours as compared to the controls.

Table 1. *Protophormia*: Stages of fragmentation

Stage	Characterized by	Time after oviposition (min at 22° C)
I	second meiotic division, karyogamy, cleavage nuclei spread through anterior 1/3 of egg	<40
II	further cleavage divisions, nuclei spread over the entire egg	40–95
III	late cleavage, nuclei reach the periplasm, pole cells appear	75–120
IV	early preblastoderm	110–140
V	late preblastoderm	140–175
VI[a]	cell borders are being formed between blastoderm nuclei	165 ± 10
VII[a]	cellular blastoderm before formation of head folds	175–210

[a] Stages VI and VII were pooled for most purposes.

The *stages selected for fragmentation experiments* are characterized by the events listed in Table 1. For easier reference the stages have been numbered from I to VII. The development times given for the different stages in Table 1 overlap in some instances because the periods allowed for oviposition (see above) varied in different series of experiments; however, the actual stage of development was ascertained in all experimental series by the double checking method.

The *percentage of eggs which continued development* after fragmentation is to some degree dependent on the stage of fragmentation, as can be seen from Table 2. Losses due to bursting range well below 5% (Table 2). Those cases in which both fragments failed to develop after fragmentation make up less than 10% of the experiments, except at stage 1 when there are very few nuclei in the egg. Failure of one of the two partner fragments to continue development is frequent only with posterior fragments of the earliest fragmentation stages (Table 2), when the posterior egg region contains no nuclei or but a few. Most important for the conclusions drawn in this paper are those cases in which both fragments continued to develop. These increase from ca. 6% for stage I to 65% for stage II, and then continue to rise until they reach ca. 90% for the latest stages.

For *assaying the patterns formed*, several criteria might be used. We tested the following four: germ band segments, neuromeres of the central nervous system, segmental tracheae and external cuticular structures. The first two criteria, although applicable to unfragmented embryos, proved impracticable with the partial embryos formed by egg fragments, because in these the furrows demarcating the individual germ band segments may be very weak, and the neuromeres could only in sections be studied with the necessary precision. Therefore, the partial larvae from egg fragments were essentially analyzed for the cuticular patterns formed, with an occasional checking of the tracheal branches. The question whether these criteria allow conclusions as to the embryonic body segments formed will be taken up in the discussion.

Table 2. *Protophormia*: Survey of fragmentations carried out, and gross results

Stage fragmentation	I		II		III		IV		V		VI+VII	
Number of eggs fragmented	51		76		111		96		72		37	
Range of levels of fragmentation (% EL)	25–70		22–71		31–76		28–66		34–59		31–74	
	n	%	n	%	n	%	n	%	n	%	n	%
Eggs burst after fragmentation	2	3.8	2	2.7	4	3.6	8	8.1	—	—	1	2.7
No development in												
both fragments	9	17.6	5	6.6	9	8.1	8	8.3	7	9.7	3	8.1
one fragment only	39	76.4	19	25.0	17	15.3	8	8.3	0	0	1	2.7
Partial larvae formed in												
anterior fragment only	37	72.5	8	10.5	9	8.1	3	3.1	—	—	—	0
posterior fragment only	2	3.9	11	14.5	8	7.2	3	3.1	—	—	2	5.4
both fragments	3	5.9	50	65.8	85	76.6	76	79.2	65	90.3	32	86.5

The Cuticular Segment Pattern and its Elements

The egg fragments produce partial larvae. These were assayed as to which elements of the normal cuticular pattern they had formed. It was therefore necessary to know the normal cuticular segment pattern of the newly hatched larva of *Protophormia* spec. This pattern (Fig. 2) consists of a linear series of 12 metameric regions. Anteriorly, the pattern starts with the "pseudocephalon". This represents the posterior parts of the head which have not undergone involution. It is followed by 3 thoracic and 8 abdominal segments. The anterior border of each segment barring the pseudocephalon is marked by a "denticle belt" consisting of several rows of small cuticular denticles. The pseudocephalon carries the opening of the preoral cavity and 2 pairs of sensory organs. From the opening of the preoral cavity a pair of tiny hooks may protude which form the anterior part of the cephalo-pharyngeal skeleton (Fig. 2) and according to Schoeller (1964) are of maxillary origin. Between them a small tooth is found which aids in hatching and is lost afterwards.

The *denticle belts of various segments* differ so that most of these can be identified even when occurring singly. For convenience they have been numbered with arabic numerals (Fig. 2). The belt of the first thoracic segment (no. 1) is ventrally much broader than those of the 2 subsequent segments, and is also characterized by the great number of very small denticles in the ventral region. The belt of the first abdominal segment (no. 4) is broader than those of meso- and metathorax (nos. 2 and 3) and expands slightly in the ventral region. The subsequent belts nos. 5–11, belonging to the abdominal segments 2–8, are progressively reduced at the dorsal side, but increase ventrally in the anteroposterior direction and tend to appear doubled there. The last abdominal segment is marked by the spiracles, the bilobate openings of the tracheal trunks which as a rule are well visible (e.g. Fig. 4b). All the pattern elements described may also appear in egg frag-

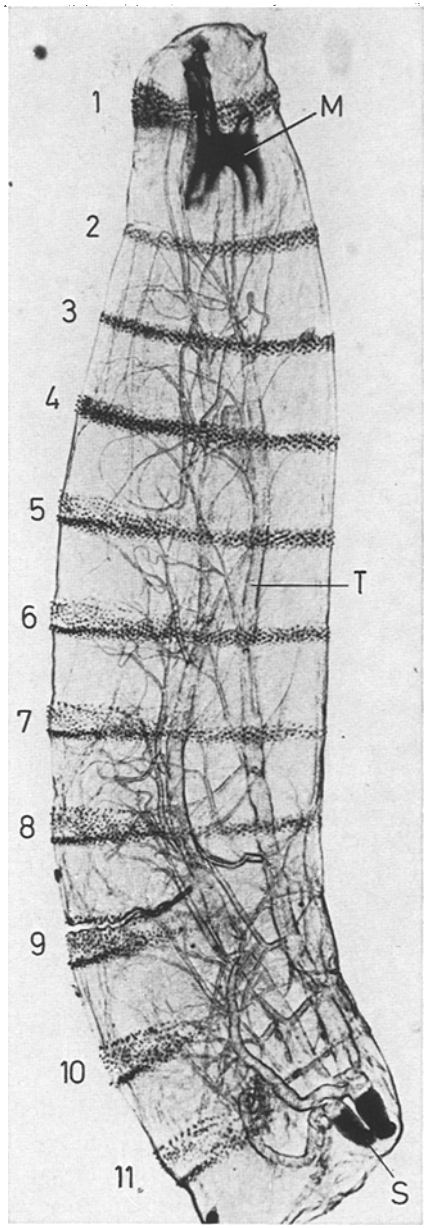


Fig. 2. Newly hatched larva of *Protophormia* spec. mounted in glycerol. The cuticle is flattened by the cover glass, and was damaged ventrally within denticle belt no. 9. Head above, ventral side to the left. Magn. ca. ×80. *M* mouth parts (cephalo-pharyngeal skeleton), *S* posterior spiracle, *T* tracheal trunk, *1–11* denticle belts from the first thoracic to the last abdominal segment

ments, although in these the cuticular structures frequently are developed weaker than normal.

Sets of Cuticular Pattern Elements Produced by Egg Fragments

The partial larvae from egg fragments may represent different regions of the complete cuticular pattern formed in the undisturbed egg. The number of segments contained in a partial larva, as judged by the cuticular structures, may vary considerably (see below). A partial larva containing only a few segments might be referred to as a small partial larva, and one containing many segments as a big partial larva. However, this terminology would be at variance with the absolute dimensions of those larvae (and their pattern elements), which may vary greatly; indeed the single pattern element, e.g. the segment as outlined by the denticle belts, tends to be bigger as the number of segments formed by a fragment decreases (e.g. see Figs. 3b, 4a). To avoid confusion, the partial pattern represented by a partial larva is therefore termed a "set of pattern elements", and this set is said to be big when it includes many elements, and small when it includes but a few elements.

When classifying the patterns formed, denticle belts of which only parts had been formed were counted as present in posterior, but not in anterior fragments.

The *sets of patterns elements formed in anterior fragments* were always found to include parts of, or the whole, cephalopharyngeal skeleton. This is always present

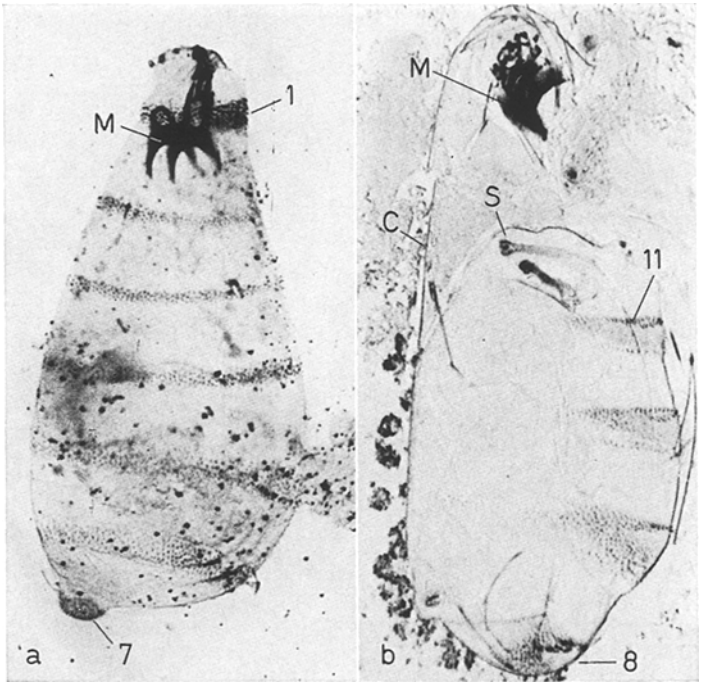


Fig. 3a and b. Cuticles of partial larvae from fragmented *Protophormia* eggs. For abbreviations see Fig. 2, *C* egg shell. Glycerol mounts. Magn. ca. ×80. a) Anterior partial larva, ventral side to the right. Denticle belts nos. 1–6 complete, parts of no. 7 present in terminal knob; pattern classified as terminating with belt no. 6. Fragmentation level 50% EL, stage VI. Posterior partner fragment see Fig. 4a. b) Cuticles of anterior and posterior fragment still enclosed in egg shell, ventral side to the right. Anterior fragment produced abnormal mouth parts and a few denticles belonging presumably to belt no. 1; classified as head structures only (*H* in Fig. 5). Posterior partner fragment formed belts nos. 9–11 and parts of belt no. 8; classified as starting with belt no. 8. The posterior partial larva has turned longitudinally by 180 degrees so that the spiracles are found next to the site of fragmentation. Fragmentation level 53% EL, stage II

if denticle belts are formed. If only one belt was formed in addition to the mouth parts, the character and distribution of the denticles were representative of the belt of the first thoracic segment (no. 1). If more belts were formed, they resembled the subsequent belts of the normal larva in normal spatial sequence. Therefore, the sets of pattern elements formed can be characterized by mentioning the hindmost belt only, for this means that all belts situated normally anterior to this particular belt have been formed too. Thus a set of pattern elements classified as terminating with belt no. 5 comprises the head structures, the three thoracic segments, and the belts of the first and second abdominal segments. The smallest set of pattern elements observed consisted of a cuticle carrying only the egg tooth. Bigger sets (Fig. 3a) may contain the mouth parts only or may terminate with any denticle belt excepting the last. Complete larvae never occurred in anterior fragments. Belts nos. 2 and 3 frequently were sclerotized so weakly that they

could be seen only with the phase contrast microscope. Partial larvae from anterior (as well as posterior) fragments contained well developed and functioning muscles.

Posterior fragments also were never able to produce the complete set of cuticular pattern elements of the normal larva. In some fragments all denticle belts were present, but the cephalopharyngeal skeleton was incomplete, indicating absence of some regions of the head. The smallest set of pattern elements formed in a posterior fragment consisted of the spiracles and only one denticle belt displaying the features of belt no. 11. Between these extreme types a complete series of sets containing 2–10 denticle belts was found to occur (Fig. 4). As in anterior fragments, those denticle belts which were formed were arranged in the normal spatial sequence as far as could be ascertained; at any rate there were no indications to the contrary. Thus a particular set of pattern elements from a posterior fragment can be characterized by indicating only the most anterior belt formed. E.g. a set classified as beginning with belt no. 5 comprises belts nos. 5–11, i.e. the abdomen from the second segment to the end.

Recognition of the pattern elements formed by posterior fragments may be hampered by the fact that the partial larvae in these fragments often take up U-shaped positions or may even revert completely within the fragment (e.g. Fig. 4). This fact which was noted already by Rostand (1927) and Nitschmann (1958) must be due to the processes of germ band extension and shortening, and/or to muscular contractions occurring later on. However, the spiracles always tell where the posterior end of the partial larva is situated.

The Size of Cuticular Pattern Sets as a Function of Level and Stage of Fragmentation

In order to recognize relations which might exist between the set of cuticular pattern elements and stage as well as level of fragmentation, the results of all experiments which yielded partial larvae were plotted against fragmentation level for each stage (Figs. 5a–f). This type of plot has been adapted from Jung (1966). Each partial larva is represented by a symbol which is located in the plot according to the fragmentation level of the egg from which it stems (ordinate), and according to its set of pattern elements (abscissa) as indicated by the first (posterior fragments) or last denticle belt formed (anterior fragments). Partial larvae from anterior fragments are symbolized by discs, those from posterior fragments by rhombs. The symbols are left open if the partner fragment from the same egg did not yield a partial larva. If the partner fragment had also produced a partial larva, the symbol is filled in.

The distribution of the symbols in the plots for different fragmentation stages (Fig. 5a–f) reveals some *general trends:*

1. The symbols are arranged from the upper left to the lower right. This means that partial larvae in long fragments on the average contain more denticle belts than those in shorter fragments. For anterior as well as posterior fragments, a positive correlation exists between number of belts and fragment size.

2. A straight curve drawn so as to be representative for the spatial distribution of the symbols for anterior or posterior fragments would be rather steeply inclined in Fig. 5b, but become progressively less steep in Fig. 5c–f. This means that the differences between the average fragment lengths required for formation

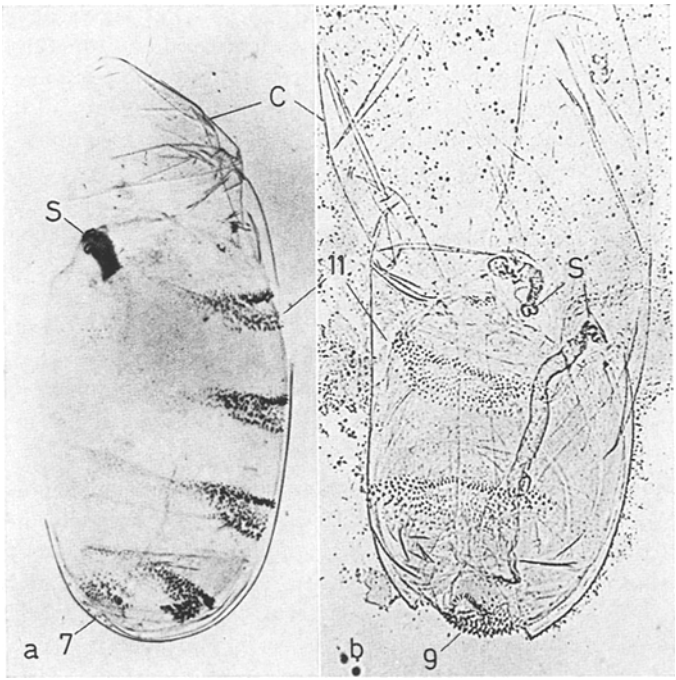


Fig. 4a and b. Cuticles formed in posterior egg fragments of *Protophormia* spec. Orientation as in Fig. 3b. Glycerol mounts, magn. ca. ×80. For abbreviations see Figs. 2 and 3. a) Denticle belts nos. 8–11 complete, parts of belt no. 7 present; pattern classified as starting with belt no. 7. Fragmentation level 50% EL, stage VI. Anterior partner fragment see Fig. 3a. b) Pattern classified as beginning with denticle belt no. 9, ventro-lateral view. Fragmentation level 43% EL, stage II. The anterior partner fragment formed mouth parts and belts no. 1+2 only

of the different sets of pattern elements decrease with increasing fragmentation stage.

3. The "clouds" of symbols for partial larvae from anterior or posterior fragments are quite separate in the plots for the early stages (Fig. 5a and b). This means that in the eggs represented by these symbols a number of belts were not formed at all.

4. With increasing fragmentation stage the symbols for anterior and posterior partial larvae draw closer together (Fig. 5e and f). This means that the discrepancy between the last denticle belts formed in anterior fragments and the first belts formed in posterior fragments of the same fragmentation level decreases with increasing fragmentation stage. This fact is borne out by a histogram representing only those eggs which were fragmented around the 50% EL level (Fig. 6). This histogram moreover shows that the reduction in the discrepancy is not due to an increase in the number of belts in either the anterior or the posterior fragment alone, but to an increase in *both* fragments: with increasing fragmentation stage the columns for anterior fragments shift to the right in the histogram, and those for the posterior fragments to the left.

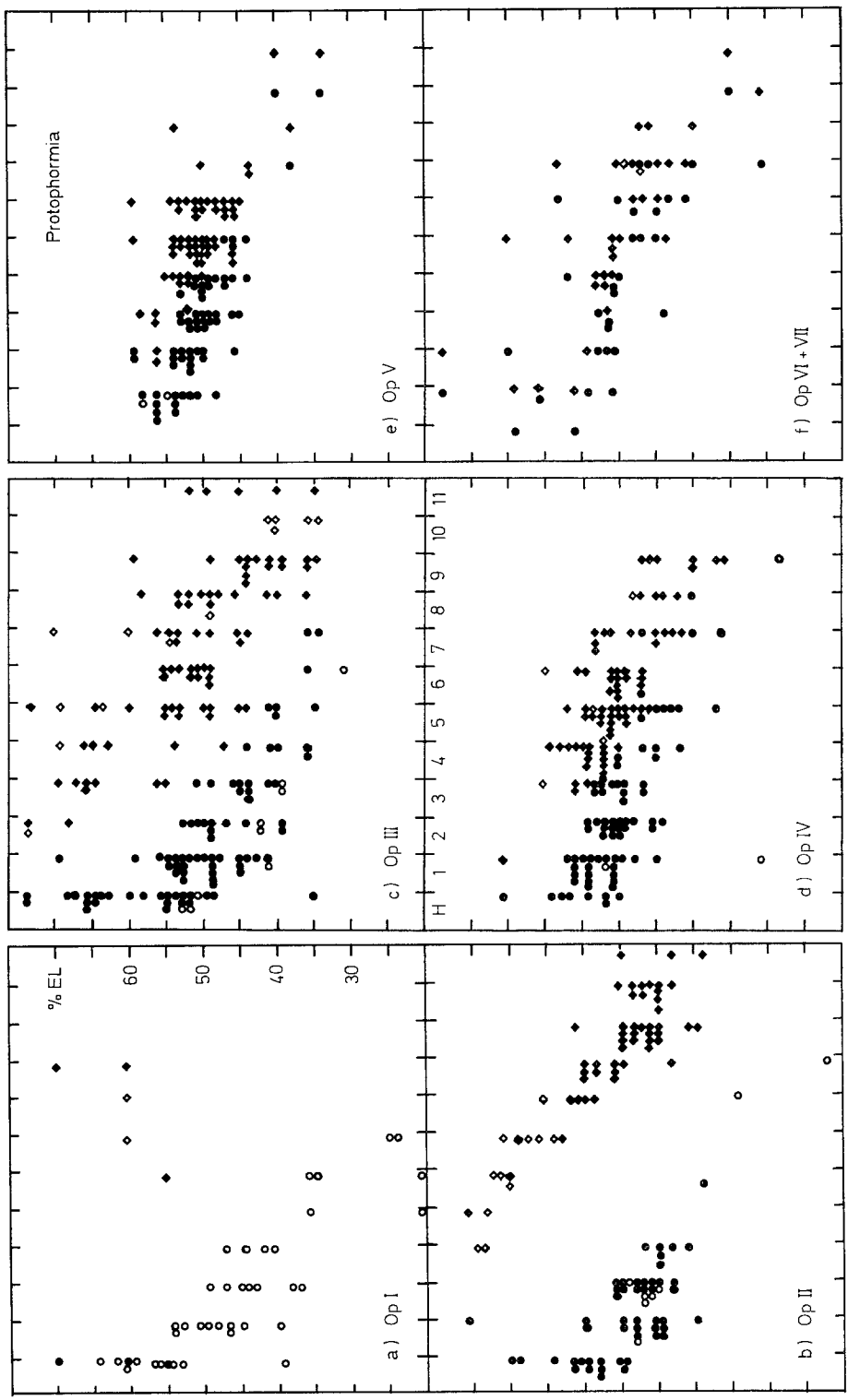
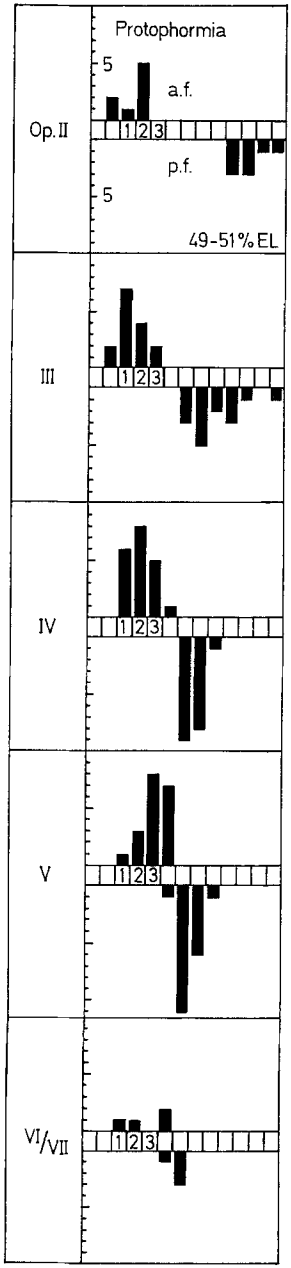


Fig. 5. Partial cuticle patterns produced by egg fragments of *Protophormia* spec. Each set of pattern elements from an anterior fragment is represented by a circle indicating (by its position) the posteriormost pattern element formed (abscissa) and the fragmentation level (ordinate). Each set from a posterior fragment is represented by a tilted square indicating the anteriormost pattern element formed (abscissa) and level of fragmentation (ordinate). Open symbols: partner fragment failed to produce larval cuticle suitable for identification. *H* head parts, *1–11* denticle belts (see Fig. 2). Diagrams a–f correspond to stages I–VI/VII



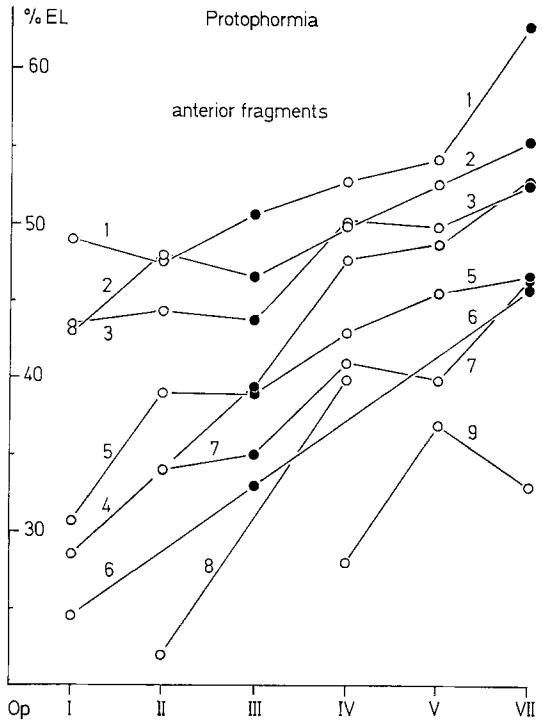


Fig. 7. Mean values of fragmentation levels required for formation of different sets of pattern elements in anterior egg fragments of *Protophormia* spec. Abscissa: fragmentation stages I–VII (excluding VI). Ordinate: fragmentation levels in % of egg length. 1–9: posteriormost pattern element of set formed. Filled circles: mean fragmentation levels for that particular set of pattern elements differ significantly (t-test, $p<0.001$) between stages III and VII

Fig. 6. Frequency distributions of partial cuticle patterns from *Protophormia* eggs fragmented halfway between the poles at different stages. Abscissae: Scales of pattern elements, denticle belts 1–3 (thorax) marked; column to the left of no. 1 represents head parts (H), columns to the right of no. 3 represent denticle belts 4–11 in that order (abdomen). Columns located above abscissa, posteriormost pattern element formed (anterior fragments, a.f.) and below, anteriormost pattern element formed (posterior fragments, p.f.). By accident, 2 cases have been omitted from stage VI/VII (a.f. 4, 6; p.f. 5, 7)

5. The variation in positions taken up by the symbols obviously varies with fragmentation stage. It is biggest in Fig. 5c indicating that in fragmentation stage III the variability of the egg's reaction to fragmentation is at maximum.

The reactions to fragmentation of the pattern forming mechanism in the egg of *Protophormia*, which have been generalized above, are now to be scrutinized

more closely under 2 aspects: the average fragment length needed to produce a particular set of pattern elements, and the relations existing between partial patterns formed in partner fragments.

Fragment Sizes Required for Different Sets of Pattern Elements

To study this aspect, the mean values of fragmentation levels at stages I–VII were calculated for fragments which had produced the same sets of pattern elements. The mean levels for different sets and fragmentation stages are presented in Fig. 7 for anterior and in Fig. 8 for posterior fragments. Corresponding values for neighbouring stages were connected by straight lines. These tend to move upwards with increasing stage in Fig. 7, and downwards in Fig. 8. This means that *in order to produce a given set of pattern elements, a fragment can be smaller when taken from an egg more advanced in development than if taken from an earlier stage. This rule holds for anterior as well as posterior fragments and is apparently an outcome of the same processes which enable a fragment of given size to produce more pattern elements with increasing fragmentation stage* [see (4) above]. It was not possible to include the standard deviations in these diagrams, but they are available (Herth, 1970). Instead, those mean values for a given set of pattern elements are marked which were shown by t-test to differ significantly between stages III and VII.

In all these cases the mean fragment length for the earlier stage proved to be greater than that for the later stage. The same tendency prevails for the remaining mean values of stages III and VII; the failure to demonstrate significance of differences in these is likely to be due to small sample size. The differences between mean values for fragmentation stages other than III and VII were not tested for significance. However, the diagrams convey the strong impression that the tendency of mean fragment sizes to decrease with increasing fragmentation stage prevails also between stages I and III.

The mean values in Figs. 7 and 8 reveal a few exceptions to the statement [see (1) above] that there exists a positive correlation between fragment size and number of belts formed. For instance the mean fragment size for pattern sets beginning with belt no. 6 at stage III is shown to be smaller than the mean fragment size for belt no. 7 (Fig. 8). However, since such exchanges in position occur erratically and are never perpetuated in neighbouring fragmentation stages, they are considered to be due to sampling errors.

Although anterior and posterior fragments share the general tendency outlined above there exist some differences in degree. Notably, the changes underlying the shifting of mean values appear to be strongest between early fragmentation stages as far as posterior fragments are concerned (Fig. 8), whereas with anterior fragments, at least for the smaller sets of pattern elements, changes tend to be more marked between later stages (Fig. 7).

If the curves connecting the mean values for anterior and for posterior fragments (Figs. 7 and 8, respectively) are projected on to each other it turns out that the mean values for anterior pattern sets ending with belt no. n and for posterior pattern sets beginning with belt no. $n+1$ come very close together for stage VII but differ increasingly with earlier fragmentation stages. This fact is exemplified in Fig. 9 using belts no. 1–4. It means that the two fragments of an egg between them should produce the complete pattern of the normal larva if fragmentation

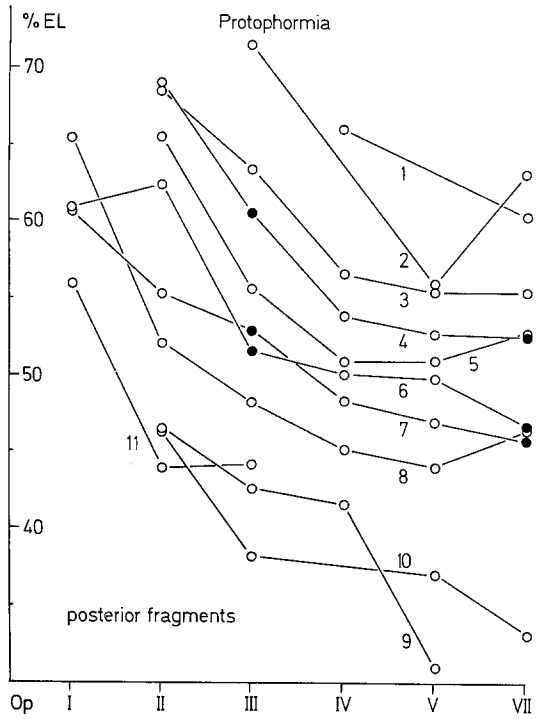


Fig. 8. Mean values of fragmentation levels of posterior fragments which produced the same set of pattern elements (*Protophormia*). Abscissa: Fragmentation stages I–VII (excluding VI). Ordinate: fragmentation levels in % of egg length. 1–10 anteriormost pattern element formed. Filled circles, see Fig. 7

is carried out at stage VII, but should fail to produce one or even several denticle belts if fragmentation occurred earlier. Consider e.g. an egg fragmented at ca. 52% EL in Fig. 9. If fragmentation is carried out at stage III, the partial larva from the anterior fragment may be expected to form only head structures and no denticle belt at all, whereas the partial larva in the posterior fragment is likely to begin with denticle belt no. 6. The belts nos. 1–5 should not be formed at all in that case. Fragmentation at stage VII, on the other hand, may be expected to yield an anterior partial larva ending with belt no. 3 and a posterior partial larva beginning with belt no. 4. These predictions will be considered in detail in the following section.

Sets of Pattern Elements from Partner Fragments

Altogether 311 eggs produced in both fragments partial larvae which were suitable for studying the pattern elements formed. Many of these failed to yield the complete series of pattern elements of the normal pattern: *some denticle belts were formed neither in the anterior nor in the posterior fragments.* As was to be expected, this failure of certain belts to be formed by a fragmented egg is correlated qualitatively and quantitatively with stage of fragmentation. This can be seen

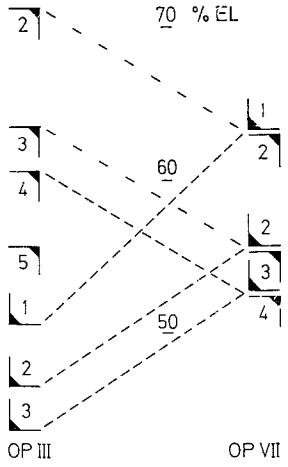


Fig. 9. Aspect of pattern formation in the egg region between 40 and 70% EL (*Protophormia*) as revealed by fragmentation experiments during stages III and VII. The lines connect the mean fragmentation levels of anterior fragments (⌊) and of posterior fragments (⌈) which next to the site of fragmentation formed the pattern element indicated (1–5). The diagram combines data selected from Figs. 7 and 8

from Fig. 10, where the eggs have been listed in 3 classes according to their levels of fragmentation. The diagram shows that in some instances 8 or 9 denticle belts failed to appear, while eggs lacking of up to 7 belts were quite frequent. The diagram indicates very clearly that, rather independently of level of fragmentation, each fragmentation stage has its characteristic frequency distribution for eggs lacking different numbers of belts. As was to be expected, the range is broadest for fragmentation stage III which showed the greatest variation in the position of symbols in the plots of Fig. 5. The essential change visible in Fig. 10 is the shifting of the frequency distribution from right to left with increasing fragmentation stage. This means that *with increasing egg age at fragmentation the number of denticle belts which fail to appear is decreasing.* The first eggs which do not lack any belt make their appearance at stage IV, but is is only at stage VI/VII that this type of result dominates.

In order to study whether the fragmentation level has some influence on the number of denticle belts which fail to be formed after fragmentation, mean values and standard deviations were calculated within each fragmentation stage for 3 classes of eggs: fragmentation level situated within the anterior half of the egg (>51% EL), fragmentation level near the middle of the egg (49–51% EL), and fragmentation level within the posterior half of the egg (<49% EL). These are listed in Table 3. When checked by *t*-test the differences which occur between the 3 classes of any single stage were found not to be significant, with the exception of stage V. For this stage, the eggs fragmented within the anterior half (>51% EL) formed significantly ($t<0.01$) fewer denticle belts than those fragmented within the posterior half (<49% EL). However, *no general and decisive influence of the level of fragmentation upon the number of denticle belts not formed due to fragmentation could be demonstrated.*

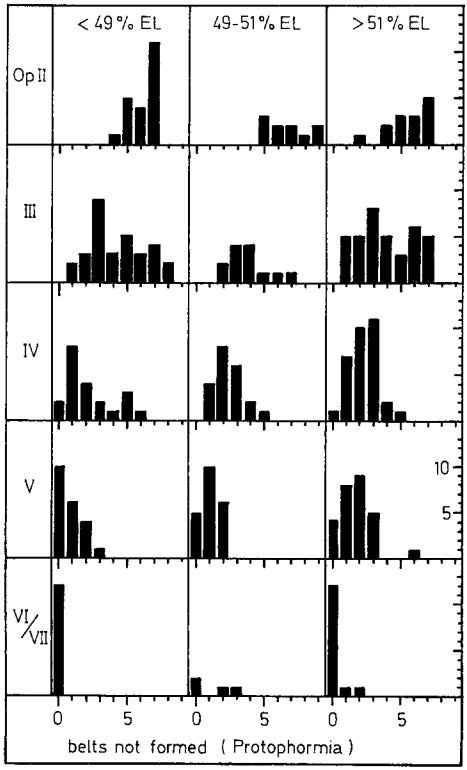


Fig. 10. Number of pattern elements (abscissa) which failed to be formed by *Protophormia* eggs fragmented during stages II–VII (ordinate). The diagram is subdivided to separate 3 classes of fragmentation levels: 49% EL (i.e within posterior egg half), 49–51% EL, and 51% EL (i.e. within anterior egg half). By accident, 2 cases were ommitted from stage VI/VII, 49–51% EL (both with all belts formed)

Table 3. *Protophormia:* Number of denticle belts not formed due to fragmentation (mean values and standard deviations)

Stage of fragmentation	Level of fragmentation		
	<49% EL	49–51% EL	>51% EL
II	6.33 ± 1.1	6.70 ± 1.56	5.57 ± 1.50
III	4.32 ± 1.97	3.85 ± 1.46	3.74 ± 1.91
IV	2.24 ± 1.48	2.43 ± 1.08	2.28 ± 1.09
V	0.82 ± 0.93	1.05 ± 0.75	1.74 ± 1.43
VI+VII	0	1.25[a]	0.21 ± 0.85

[a] 4 cases only.

II. Experiments on *Drosophila* Eggs

By a fragmentation technique for single eggs based on the same principle as described for *Protophormia*, but adapted for use with smaller insect eggs, a number

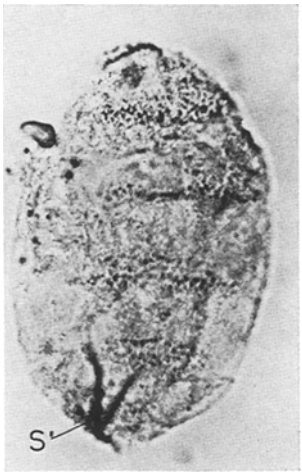


Fig. 11. Posterior partial larva from an egg of *Drosophila melanogaster* fragmented near 50% EL at the cellular blastoderm stage. The cuticle pattern starts with parts of belt no. 7. The anterior partner fragment had formed belts nos. 1–6. Magn. ca. ×140. *S* spiracles

of wild-type *Drosophila melanogaster* eggs have been fragmented. Losses due to bursting were much heavier than in *Protophormia*, and externally intact fragments frequently failed to continue development. Therefore, even with the least sensitive stage tested (cellular blastoderm) only some 10% of all fragments yielded partial larvae suitable for analysis of the cuticular pattern. The experiments were carried out during 3 stages of development: cleavage before formation of pole cells, stages between pole cell formation and formation of blastodermal cell walls, and cellular blastoderm. The fragmentation level was close to 50% EL. Eggs fragmented during cleavage did not in any case produce partial larvae; all fragments either burst or failed to develop further. From the fragmentations carried out during preblastoderm stages, altogether 8 eggs were obtained with partial larvae in both fragments. *In all these eggs either 1 or 2 cuticular segment borders were lacking.* Of the eggs fragmented after formation of blastodermal cell borders, a total of 6 formed partial larvae with cuticles (Fig. 11) in both partner fragments. *In all cases these summed up to the complete pattern, i.e. no cuticular segment border failed to be formed.* These results, although of a very preliminary character, are *compatible with the results in Protophormia* and the conclusions drawn from these, and therefore are included here.

III. Discussion

As documented in the previous sections, the patterns of larval cuticle formed by *Protophormia* egg fragments reveal the following reactions of the developing system when fragmented at different stages before and during blastoderm formation:

1. Sets of denticle belts (pattern elements) formed by anterior fragments always include belt no. 1 (foremost belt of the pattern), those formed by posterior fragments always include belt no. 11 (hindmost belt). Additionally, adjacent belts of

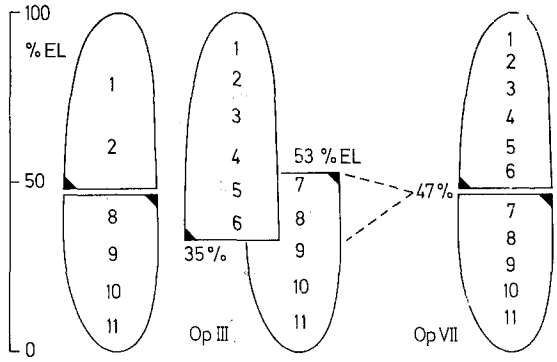


Fig. 12. Diagram illustrating the essential effects of fragmentation upon pattern formation in the *Protophormia* egg. *OP III* and *OP VII:* fragmentation stages (see Table 1), *1–11:* denticle belts (pattern elements) (see Fig. 2). Left egg shows gap in series of pattern elements caused by early fragmentation. Right egg was fragmented late and produced the full series of pattern elements ("mosaic" type reaction). In order to produce by fragmentation at stage III the sets of pattern elements shown for stage VII (1–6, 7–11), *both* anterior and posterior fragments have to be longer (see middle of figure). Based on data from Figs. 7 and 8

the normal pattern may be formed in one or both fragments depending on fragment size and stage of fragmentation.

2. After fragmentation during early stages of development (cleavage) a number of pattern elements (denticle belts) fail to be formed altogether. Fragmentation thus causes a gap in the belt pattern whose extent and position within the pattern depend upon level and stage of fragmentation (Fig. 5b, c).

3. With increasing egg age at fragmentation, this gap in the cuticular pattern becomes progressively smaller (Fig. 5d, e, Table 3); it is missing altogether from most eggs fragmented during or after formation of blastodermal cell walls (Fig. 10).

4. With the fragmentation levels studied, this reduction of the gap in the cuticular segment pattern is due to formation of bigger sets of pattern elements in *both* egg fragments. I.e., on the average an anterior or posterior fragment of given size will produce more pattern elements if separated from the remainder of the egg at a later stage than if separated early (Fig. 6).

5. Conversely, in order to produce a pattern set of given size (e.g. denticle belts no. 1–4), a fragment needs to be bigger when separated early and may be somewhat smaller when separated later on. This rule applies to both anterior and posterior fragments (Fig. 7–9).

These results are exemplified by Fig. 12 (see legend). At first sight they might be interpreted as *due to suppression of locally present morphogenetic information by some kind of damage inflicted on the developing system during the process of fragmentation.* The system then would have to be more sensitive to such damage during the early phases of its development than later on. The architecture of the egg system, which is very labile and not well supplied with nuclei at the beginning of development, would favour such an interpretation. However, several observations indicate that this interpretation is too simple. If the gap in the pattern of denticle belts is caused

by lability of the egg architecture, this lability should also frequently cause failure of egg fragments to continue development altogether. Thus the extent of the gap resulting from fragmentation at a given stage should be correlated quantitatively with the percentage of fragments which cease to develop after the operation. This should apply to both fragments of an egg, since the apparent "loss" of denticle belts resulting in the gap is shared by both fragments: both fragments would have done better, at least statistically, if separated later on (see 4 above). However, Table 2 reveals no such correlation: the result "no development in both fragments" occurs with approximately constant frequency over the stages II–VII and is more frequent only for stage I. The decrease of cases in which only one fragment failed to develop corresponds somewhat more closely to the expectation; this, however, might be due to the degree of nucleation of smaller fragments which increases between stages I and IV and thereby reduces the chance of a fragment to receive no nuclei, or an insufficient quantity of nuclei. Moreover, direct observation in *Protophormia* confirmed the finding of Nitschmann (1961) that after early fragmentation no damage is visible in the blastoderm bordering the site of separation (Fig. 1c). Likewise, formation of intact pattern elements (denticle belts) quite close to the site of fragmentation, and the rather equidistant distribution over the entire fragment length of whatever denticle belts have been formed *all speak against suppression of locally present morphogenetic information by the damage inflicted during fragmentation.* Finally, the fact that the appearance of a similar gap in the segment pattern of the leaf hopper *Euscelis* (Fig. 14) can be prevented by an additional operation likely to inflict still more mechanical damage on the system (Sander, 1960) calls for alternative explanations of the results described in this paper.

An interpretation which is not based on destruction of locally preformed morphogenetic information might start from the observation that with anterior as well as posterior fragments the mean fragment size required statistically for formation of any particular set of pattern elements *decreases* with age (see Figs. 7 and 8). This may be explained on the assumption that until blastoderm formation anterior and posterior egg regions influence each other mutually with respect to pattern generation, and that fragmentation interrupts the normal interaction. The gap observed in the cuticular segment pattern would then not be due to damage inflicted during fragmentation, but to *prevention of any further interaction between the egg regions separated by fragmentation. This of course implies that the morphogenetic information for those pattern elements which fail to appear in fragmented eggs does not need to be locally present at the time of fragmentation. It rather may fail to become located correctly or even to become specified at all because the machinery required for this is interrupted.*

If this assumption is basically valid, the data presented in this paper suggest *interaction between anterior and posterior egg regions for pattern formation.* For this interaction, 2 types of model may be considered, which are referred to as "qualitative" and "quantitative". The "qualitative" type may best be explained using Figs. 9 and 12. Fig. 12 (middle) shows that posterior fragments of stage III averaging 53% EL are able to produce a partial pattern starting with element no. 7. Smaller fragments (47% EL) separated at this stage are on the average unable to form element no. 7 (left egg). However, such fragments do form element

no. 7 if separated later on, at stage VII (right egg). Accordingly, *some precondition for the formation of this element must have moved backwards* by some 6% EL between these 2 stages, as indicated by the upper dashed line. The same type of argument can be applied to the anterior fragments shown in Fig. 12. Anterior fragments separated at 47% EL during stage III produce on the average only elements no. 1+2 (left egg). In order to produce element no. 6, they need to be much bigger (fragmentation level ca. 35% EL, middle egg). If separated at stage VII, however, the smaller fragment (47% EL) is now able to form elements no. 1–6 (right egg). Accordingly, *some precondition necessary for element no. 6 must have moved anteriorly* in the egg between stages III and VII (lower dashed line), thereby enabling an anterior fragment of the 47% EL level to form this element (and elements 3, 4 and 5 as well). *This type of argument can be extended to any one of the pattern elements from no. 1 down to at least no. 8.* Fig. 9 can be used to illustrate this for elements no. 2 and 3. Their formation in a posterior fragment is thought to be dependent upon an anterior prerequisite which apparently moves backwards during development, and their formation in an anterior fragment requires a posterior prerequisite which apparently moves anteriorly. Pattern formation then could be considered as due to 2 sets of preconditions for the different pattern elements, an anterior and a posterior set. The "*qualitative*" *type of model* would embody qualitatively different preconditions for the different pattern elements within each set, i.e. the anterior precondition for element no. 2 would differ *qualitatively* from that for element no. 3, and so would the posterior prerequisites for elements no. 2 and 3. In the "*quantitative*" *type of model,* the preconditions for the different pattern elements within each set are thought to represent merely local differences in *quantity* of some essential component(s) of the pattern forming system. The results described in this paper *do not permit discrimination between these two types of model.* However, on the basis of results obtained in other insect species the "quantitative" type would appear to be more suitable for the interpretation of pattern formation in higher dipterans, too.

A "*quantitative*" model has been used to interpret the multitude of results obtained by different operations on the leaf hopper egg (Sander, 1960). It employs two gradients, one originating from the anterior, the other originating from the posterior border of the pattern forming egg region; the specifity of the morphogenetic information required to trigger the genes for different pattern elements is ascribed to the ratio "level of anterior gradient/level of posterior gradient" which of course would change continuously along the axis between the 2 maxima or regions of origin of the gradients. Strongest support for a "quantitative" type of model comes from experimental demonstration of the polarity reversal predicted for a certain type of experiment (Sander, 1961). The data reported in this paper do not per se require a "quantitative" model as complex as that inferred form the leaf hopper experiments. From a purely formal point of view they might be interpreted by assuming that a single gradient is build up from a limited quantity of "something" so as to reach different threshold values along the longitudinal axis of the system, and thereby to cause the cells located at or between these thresholds to react differently. If the "something" is produced near one egg pole, fragmentation of the system before the gradient has reached its final shape would lead to a surplus quantity in one fragment, and to a deficit in the other. The resulting drop of gradient level at the site of fragmentation would account for the failure of a number of pattern elements to be specified altogether. However, such a model in *Protophormia* is unlikely to function on a simple physico-chemical basis. It will require further elaboration possibly along the lines suggested by Lawrence *et al.* in their "homeostatic model" and/or the models derived from the results in *Euscelis.*

Both "qualitative" and "quantitative" models as outlined above involve real or apparent movements of morphogenetic information (see Figs. 7–9). One might ask whether these movements could be due to the "spasms" or "oscillations" as observed in other dipteran eggs (e.g. Counce and Ede, 1956; Kinsey, 1967). No such movements have been observed in *Protophormia* so far, and in any case it is difficult to imagine how these movements could account for antagonistic transport effects within the same egg region as suggested by Fig. 9. However, slow and unidirectional movements of egg materials carrying morphogenetic information might account for the stage-dependent differences in the *rate* of decrease of mean fragment sizes required for different sets of pattern elements as evident from the slopes of curves in Figs. 7 and 8. A slow backward movement of such materials in the anterior egg half between stages I and III (or IV) e.g. might partly be responsible for the slow ascent of curves 1–3 in Fig. 7 and at the same time cause the very steep descent of most curves in Fig. 8 between stages I and III. The wide variety of results obtained for stage III (see Fig. 5c) may indicate that between stages II and IV some rather rapid changes take place in the developing system, with some difference in timing in different individuals.

On turning to *other insect species* we find that the *trends revealed by the partial cuticle patterns from fragmented Protophormia eggs (see 1–5 above) have been noted before with respect to the pattern of germ band segments* in several species. They were documented extensively in the leaf hopper *Euscelis* (Sander, 1959–1962) and in the Bean Beetle *Bruchidius* (Jung, 1966); results from some other species, e.g. the Chironomid midge *Smittia* (Sander, unpublished) and the beetle *Necrobia rufipes* (Trendelenburg, 1971) indicate similar reactions to fragmentation. It is rewarding to compare these results, but before this can be done the question arises whether the cuticular segment pattern of *Protophormia* studied in this paper is strictly comparable to the pattern of germ band segments studied in other species. Nitschmann (1958) who made the first extensive series of fragmentation experiments on eggs of higher dipterans answered this question in the affirmative by speaking simply of "segments". Some doubts are raised concerning this practice by the findings of Davis, Krause, and Krause (1968) who reported that egg fragments of *Calliphora* cultivated *in vitro* may produce more neuromeric segments than cuticular segments; this might mean that the failure of fragmented *Protophormia* eggs to produce the complete segment pattern might be restricted to the cuticular structures whereas the neuromeres, and consequently the germ band segments, would have been more numerous and even might have represented the complete pattern. However, several facts render this possibility unlikely. Only 2 will be mentioned here: in partial larvae from early fragmentations, when many cuticular segments fail to form, the discrepancy between cuticular and neuromeric pattern should have been so big that it probably would not have escaped observation. The effect described by Davis *et al.* may be observed also in fragments separated at post-blastoderm stages when *Protophormia* eggs produce the complete cuticular pattern after fragmentation. This indicates that the effect might be due to suboptimal *in vitro* conditions which would affect the surface of the explant (i.e. the epidermis) more strongly than the internal organs; an assumption supported by the abnormal course of external segment formation described by these authors. Therefore, in the subsequent discussion the pattern of cuticular segments

in partial larvae of *Protophormia* is considered representative of the pattern of germ band segments originally formed. Should this prove incorrect, then in the following comparison merely "germ band segment" should read "cuticular segment", since all experience indicates that in leaf hopper and beetle eggs each germ band segment in an egg fragment will produce its share of the cuticular pattern if given the chance.

In order to facilitate comparison between different species, two diagrams (Fig. 13 and 14) have been drawn from the data of Jung (1966) on *Bruchidius* and Sander (1959 and unpublished) on *Euscelis*; a similar diagram has been prepared for the Chironomid midge *Smittia* (Sander unpublished). Fig. 13, which is comparable to Fig. 6, shows that in the Bean Beetle early fragmentation causes a *gap in segment pattern.* In eggs fragmented approximately halfway between the poles, the reduction of this gap with increasing egg age is due, as in *Protophormia,* to the formation of additional segments in both anterior *and* posterior fragments. Fig. 14 represents the frequency distribution of the number of segments not formed after fragmentation in eggs of the leaf hopper *Euscelis;* as in *Protophormia* (see Fig. 10) this number *decreases with increasing egg age* at fragmentation. The really interesting aspect of these comparisons, however, is revealed when the *stages* used in the different species are considered. Stage III in *Protophormia* corresponds roughly to stage PC in *Bruchidius* and to stage CL/BL in *Euscelis.* The stage marked PG in *Bruchidius* and *Euscelis* is already much more advanced than stage VII in *Protophormia.* Scrutinizing Figs. 6, 10, 13 and 14 with this in mind one realizes that *formally comparable processes involved in generating the pattern of body segments appear to take place much earlier in the development of Protophormia than in the development of the other two species.* A "mosaic type reaction" to fragmentation, i.e. formation in partner fragments of partial patterns which taken together represent the whole pattern, occurs in *Protophormia* on completion of the blastoderm. In *Smittia,* however, this was not observed until a short time before germ anlage formation (Sander, unpublished) and the *Euscelis* egg reaches mosaic status (with reference to the longitudinal pattern) only at the time of germ anlage formation. This difference in the timing of otherwise comparable processes serves to reinforce the opinion held by many authors that *a gradual transition exists between the extremes of "mosaic type" and "regulative" embryonic development,* and that accordingly *"the two ends of the spectrum are simply manifestations of a time differential in the determination processes"* (Counce, 1961). A scale of transitions between determinate and indeterminate insect egg types was established on morphological evidence by Seidel (1924) and later on improved by Krause (1939) who incorporated experimental results. On this scale the higher dipterans represent the extremes of determinate development, whereas hemipterans and beetles range in the middle of the scale close to each other. The lower dipterans, e.g. *Smittia,* should be placed somewhere between higher dipterans and beetles. *The reactions of these egg types to fragmentation as discussed above fit very well into this scheme. What is surprising, however, is the degree of "indeterminateness" which is observed even in the presumably most determinate insect egg type,* that of the higher dipterans.

The notion of strict mosaicism prevailing in the embryogenesis of higher dipterans (see Schleip, 1929) has been challenged recently by some authors

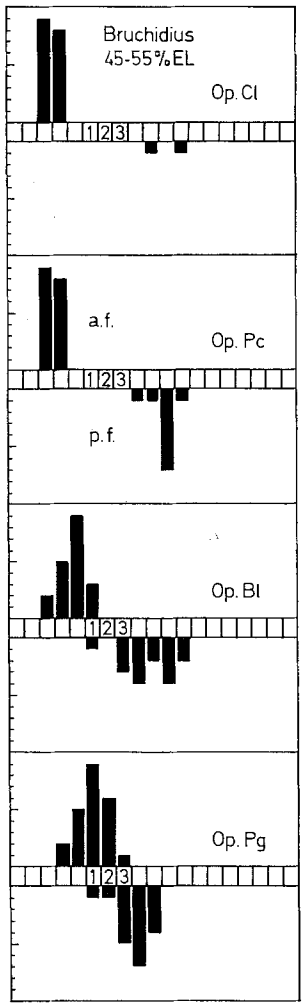


Fig. 13

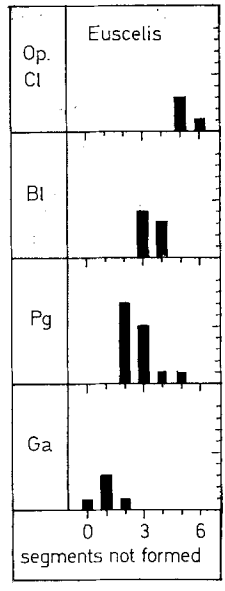


Fig. 14

Fig. 13. *Bruchidius obtectus* Say (Bean Beetle), diagram based on data from Jung (1966). The eggs were fragmented by ligature at 45–55% EL and the fragments scored for the germ band segments formed. The diagram corresponds to Fig. 6 of this paper. Stages: *Cl* cleavage (*Fu*), *Pc* preblastoderm with pole cells (*Pz*), *Bl* uniform blastoderm (*Blm*), *Pg* blastoderm thickened laterally (Vorkeimanlage, *Vka*)

Fig. 14. *Euscelis plebejus* Fall. (leaf hopper), diagram based on data from Sander (1959 and unpublished). The eggs were fragmented behind 40% EL by ligature and the fragments scored for the germ band segments formed. The diagram corresponds to Fig. 10 of this paper. Stages see Fig. 13, *Ga* germ anlage (Keimanlage, *KA*)

(e.g. Counce and Selman, 1955; Nitschmann, 1959; Counce, 1961) but was upheld by others. Anderson (1966) argues that the work of Hathaway and Selman (1961) "supports the classical view that the presumptive areas of the blastoderm are determined and regionalized as a mosaic of precursors in the fertilized egg"

although these authors state only that no departure from mosaicism was noted in their results. Slight "regulations" observed by these and other authors are considered by Anderson as not irreconcilable with ooplasmic segregation because they occur "within the individual larval presumptive areas"; accordingly, "there is no evidence to suggest a regulative switch in the fate of any one area to that of another". If, for reasons which have been set forth elsewhere (Sander, 1971), the term "regulative" is omitted from this statement, the experiments described by Nitschmann (1959, 1961) and by the present authors provide this evidence. Nitschmann showed that presumptive blastoderm cells of the equatorial region in *Calliphora* may be induced to become secondary vitellophages (see Krause and Sander, 1962), and that one and the same egg region may be able to form quite different body segments. The latter conclusion is strongly supported by our experiments on *Protophormia* as will be realized on studying Fig. 12. In the experimental situation depicted there the egg region between ca. 35 and 53% EL will form pattern elements (denticle belts) no. 6 and 5 when included in the anterior fragment during stage III, but will form pattern elements no. 7 and 8 when included in the posterior fragment; after fragmentation during stage II the differences, according to Fig. 6, would be still more striking. *Considering that no sizeable amount of blastoderm cells is lost in the fragmentation process, the situation illustrated in Fig. 12 is good evidence for a switch in the fate of one area to that of another. If the Protophormia egg were a mosaic type system, any region should form the same pattern element(s) irrespective of its connections to (or separation from) a neighbouring region. As shown by our experiments, the system does not fulfil this expectation at the beginning, but only when it becomes subdivided into many cells.*

It should be pointed out again here that production of defective patterns by a fragmented or otherwise maltreated developing system *per se* is not a proof of mosaic status. To argue to the contrary is to be unaware of the (frequently encountered) possibility that those pattern elements which were formed after interference with the system may not have been formed at all by those regions of the system which produce them in normal development (see Sander, 1971, Fig. 6).

As the dipteran egg, according to the evidence presented above, does not yet at oviposition represent a mosaic of determinants for various pattern elements, the body pattern which finally becomes visible must be specified epigenetically. Some aspects of models which might account for this process have been discussed above. They ascribe pattern formation in the middle region of the egg system to antagonistic influences exerted by anterior on posterior regions, and *vice versa*. In this respect they correspond to conclusions derived from experiments on leaf hopper (Sander, 1959–1962) and Chironomid eggs (Yajima, 1960; Kalthoff and Sander, 1968). They are, however, in conflict with the widespread opinion that regionalization in insect eggs should be due to a differentiation centre located somewhere in the middle of the pattern forming region. Such a centre was first proposed by Seidel (1934) as prerequisite for formation of the germ anlage, and later on endowed with the function of spreading out the segment pattern over the future embryo. The latter conception appears to have been sparked by results of Schnetter (1936) on honey bee eggs which, however, were confined to posterior egg fragments. On comparing Fig. 8 to Figs. 7 and 9 of the present paper one will immediately realize what dangers of misinterpretation are involved in neglecting

the results of the corresponding anterior fragments. But quite apart from this, the concept of segment anlagen spreading out from a single differentiation centre, located in the prothorax or elsewhere, is unable to account for the results obtained with *Protophormia*. Our results also provide no indication for a differentiation centre necessary for development beyond the blastoderm stage as postulated by Anderson (1961) for *Dacus tryoni;* a corresponding effect might show up in smaller fragments of *Protophormia* eggs than those studied by us, but in this case it would certainly not be associated with the prothoracic segment as concluded by Anderson.

Whatever the mode of regionalization in the developing cyclorrhaphan egg, eventually each cell or group of cells must receive a specific instruction as to which structures of larva or adult it has to produce. The question *at what time the cells accept this order irrevocably*, i.e. appear to have undergone cell determination, cannot be answered by experiments of the type employed in this study. However, cell determination is unlikely to become firmly established before pattern determination (regionalization) has proceeded to the point from which the system reacts as developmental mosaic. Since in *Protophormia* and *Drosophila*, according to our experiments, this state of the system is not reached before the blastoderm stage, cell determination should not be completed earlier (and could not be, if separate cells are required); if cell determination is a short-time process, it may even not begin before cell walls appear in the future blastoderm. Two recent results on *Drosophila* eggs provide some indications concerning this timing, and these are compatible with our conclusions. Zalokar (1971) on the basis of nuclear transplantation experiments considers the cleavage nuclei in *Drosophila* to be equal and omnipotent up to at least the 256-nuclei stage, whereas Chan and Gehring (1972) find restrictions in the developmental capacities of cultivated blastoderm cells, and these are correlated to the region (anterior or posterior egg halves) from which the cells originated.

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