

Manipulating the anteroposterior pattern of the *Drosophila* embryo

HANS GEORG FROHNHÖFER, RUTH LEHMANN
AND CHRISTIANE NÜSSLEIN-VOLHARD

*Max-Planck-Institut für Entwicklungsbiologie, Abteilung III (Genetik),
Spemannstraße 35, 7400 Tübingen, Federal Republic of Germany*

INTRODUCTION

The establishment of pattern and polarity in the insect egg has been studied intensively using a variety of experimental approaches. *Drosophila*, while the system of choice for genetic analysis of pattern formation has been rather neglected as an experimental organism and species with longer developmental time and larger eggs were preferred in classical studies. Among the dipteran insects, midges such as *Chironomus* and *Smittia* with their transparent chorion and synchronous development were found more rewarding. The classical methods of ligation, puncture, transplantation, destruction or removal of material, and centrifugation were applied to eggs of a variety of insect species. Although the degree of response to experimental manipulation was found to be widely different, there were similarities in the type of abnormal patterns produced by the various treatments which suggested more general conclusions:- the anteroposterior pattern is probably controlled by two centres of activity, localized at the anterior and posterior egg pole respectively, with a long-range effect on the entire egg axis (reviewed by Sander, 1976).

One of the earliest clear indications of an 'activation centre' localized at the posterior egg pole came from ligation experiments on the egg of the dragon fly *Platycnemis* (Odonata) (Seidel, 1929). Removal of the posteriormost 10 % of the egg by ligation in early cleavage stages prevents embryonic development in the larger anterior portion of the egg. If ligation is done somewhat later, partial embryos develop in the anterior portion suggesting that a time-dependent spreading of some factors is required for pattern formation in anterior egg regions. The organizing influence of material localized at the posterior pole has best been shown by Sander (1959, 1960) in the leaf hopper *Euscelis* (Homoptera). This insect has an unusual cytoplasmic inclusion, a ball of symbiotic bacteria located at the posterior pole. Because of the fortuitously low turgor of the egg, this ball of symbionts together with adhering posterior cytoplasm can be easily pushed around in the egg without needing to penetrate the egg membranes. Sander showed that

Key words: *Drosophila*, anteroposterior pattern, cytoplasmic transplantation, induction.

posterior pole material induces abdomen formation and acts as a polarizing source at ectopic regions.

Ligation experiments on *Euscelis* suggested the existence of a similar anterior organizing centre, since not only are the developmental capacities of anterior fragments dependent on the time of contact with material from the posterior part, but also the degree of anterior development reached in the posterior fragment is reduced by early separation from the anterior end. Ligation experiments have been carried out in a number of holometabolous insects. Interruption of signal traffic along the anteroposterior egg axis appears as a 'gap' in the pattern: segments close to the constriction site are missing and therefore the anterior and posterior parts do not add up to a complete pattern. A 'gap' phenomenon was observed in most species tested including coleopterans (*Bruchidius*, Jung, 1966; *Callosobruchus*, van der Meer & Miyamoto, 1984) and dipterans (*Protophormia*, Herth & Sander, 1973; *Drosophila*, Schubiger, Moseley & Wood, 1977), supporting the generality of the principle.

More direct evidence for the existence of anterior factors in addition to posterior ones came from experiments with dipteran eggs. Yajima (1960, 1964) and Kalthoff and co-workers (Kalthoff & Sander, 1968; reviewed by Kalthoff, 1979) applied various treatments like u.v. irradiation and centrifugation to eggs of midges of the species *Chironomus* and *Smittia* respectively. They succeeded in producing embryos with double abdomens or double heads as well as some other types, such as embryos that develop a normal pattern in reversed orientation in the egg case. Double abdomen embryos comprise two hindends arranged in mirror-image symmetry. A variety of treatments of the anterior pole, such as u.v. irradiation, extraovate formation following pricking and RNase application cause double abdomen formation specifically (Kalthoff & Sander, 1968; Schmidt, Zissler, Sander & Kalthoff, 1975; Kandler-Singer & Kalthoff, 1976). These spectacular monsters were interpreted by Kalthoff as resulting from the destruction of an 'anterior determinant' composed of ribonucleoprotein. The double head embryos obtained by posterior u.v. irradiation in *Chironomus* (Yajima, 1964) suggest an analogous 'posterior determinant'.

In *Drosophila*, similar attempts to change the somatic pattern by experimental treatment did not give very informative results. In contrast, the elegant transplantation experiments of Illmensee & Mahowald (1974) provided evidence for factors localized at the posterior pole that are required for germ cell formation. The pole cells, the precursors of the germline cells, are the first cells to form in the *Drosophila* embryo. A specialized cytoplasm localized in the posterior egg region is included in the pole cells during their formation. The polar granules that characterize the pole plasm (Mahowald, 1974) soon provoked speculations as to their role in pole cell determination. Illmensee & Mahowald (1974) could induce the formation of functional pole cells at an ectopic egg region by transplantation of pole plasm to the anterior tip of the egg. Unfortunately, the effect of pole plasm on the somatic organization of the host embryos has not been examined.

The first deliberate attempts to study the effect of manipulations on the somatic pattern of the *Drosophila* embryo were made by Bownes (Bownes & Sang, 1974; Bownes, 1976; Bownes & Kalthoff, 1975). Following egg puncture and microcautery they examined abortive development of the unfixed and unstained embryos using Nomarski Interference optics. Owing to high mortality and a rather heterogenous spectrum of defects obtained after puncturing the egg at various sites, these studies were not very rewarding. In particular, the spectacular double abdomens of Yajima and Kalthoff could not be obtained in *Drosophila* with similar treatments (Bownes & Kalthoff, 1975; Bownes, 1976). On the other hand, the phenotypes of the maternal effect mutant *bicaudal* (Bull, 1966) are very similar to the double abdomen embryos of other species, strongly suggesting that similar pattern-forming mechanisms operate in these systems (Nüsslein-Volhard, 1977, 1979).

OBSERVATIONS

In the course of our studies on mutant *Drosophila* embryos we were encouraged to repeat and extend some of the older experiments. Two simple operations were performed: removal of cytoplasm from various egg regions and transplantation of cytoplasm from various egg regions to the anterior pole. As previous attempts using the same operations proved difficult, a few remarks on the technique appear appropriate. Pricking of eggs that have not been dried to reduce their turgor causes leakage of about 5 % of the egg content. If this material is left adhering to the embryo during development, the embryo almost inevitably dies. Careful and, if necessary, repeated removal of the leaking cytoplasm allows most of the embryos to survive to the larval stage of differentiation. Late leakage often causes a specific lethal syndrome which is reminiscent of the phenotype of the neurogenic mutants (Lehmann, Jiménez, Dietrich & Campos-Ortega, 1983) in that only dorsal cuticular structures are formed while ventral and lateral epidermis is completely absent. In order to avoid leakage in the transplantation experiments, the recipient embryos were dried briefly. About 2 % of the egg volume was transplanted. All operations were performed with embryos in the early cleavage stage before pole cell formation. The experimental designs are illustrated in Fig. 1.

Experiment A

Removal of cytoplasm from the anterior tip causes a strong reduction in the occurrence of head structures. The results reported in Table 1 show that those structures usually derived from the anteriormost head region, the labrum and the inner parts of the cephalopharyngeal skeleton, were always lacking. In some embryos the head was completely absent and the thorax reduced to one or two instead of three segments. In addition, telson structures normally formed at the posterior end of the embryo such as analplates, tuft and characteristic sensory cones were duplicated at the anterior. During gastrulation the cephalic furrow and the posterior dorsal fold normally appearing at 67 % and 40 % egg length

respectively, were shifted towards the anterior (Fig. 2). The cephalic furrow may disappear completely. Frequently instead, a posterior midgut invagination is observed at the anterior pole.

Experiment B

A very similar, if not identical, range of phenotypes is obtained by transplantation of posterior pole plasm into the anterior tip of the embryo. In the experiment of Table 1, about half of the treated embryos had reduced head structures and some showed duplications of telson structures at the anterior (Fig. 3). The frequency of posterior duplication declines sharply with the age of the embryo (data not shown), which may explain the difference in frequency of embryos with reduced heads in experiments A and B. Since for transplantation embryos must be dried, they are on average older than those that just have to be pricked.

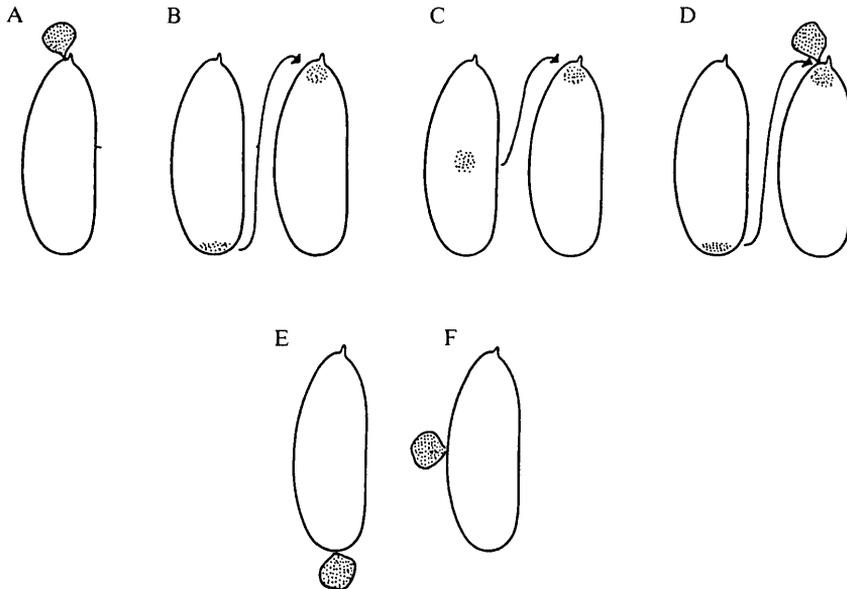


Fig. 1. Protocols of pricking and transplantation experiments (see Table legends). Embryos were collected from Oregon-R wild-type females for 1 h at 18°C, dechorionated with Klorix and mounted in suitable orientations on a coverslip spread with glue. For pricking (experimental designs A, D, E and F), eggs were covered immediately after mounting with Voltalef 10S oil and pricked with a glass needle. After about 15 min, the extruded cytoplasm was removed using a wide glass capillary. If necessary, this clearing treatment was repeated. For cytoplasmic transplantation (experimental design B and C) the recipient embryos were dried briefly under a fan to avoid leakage. Cytoplasm was collected from several donor embryos and distributed into approximately the same number of recipient embryos. In experiment D, the ratio of donors to recipients was between one and two. After the experimental treatment, embryos were left to develop in a moist chamber. Unhatched larvae were removed from the vitelline membrane using a glass needle and, together with the hatched larvae, mounted in Hoyers medium diluted 1:1 with lactic acid. After clearing at 60°, the embryos were screened for the presence of cuticular markers (Lohs-Schardin *et al.* 1979).

Table 1. Induction of posterior structures at the anterior of the *Drosophila* embryo

Experimental design*	Number of surviving embryos†	Embryos with the pattern‡: (% of surviving embryos)				
		normal	small head	posterior duplications		
				telson	asymmetric DA	symmetric DA
A	46	0	93	7	0	0
B	90	54	43	2	0	0
C	32	94	6	0	0	0
D	61	0	20	34	30	16

* See Fig. 1.

† In general 50 to 80 % of all treated embryos.

‡ For the description of the patterns see text and Figs 3 and 4.

DA = double abdomen.

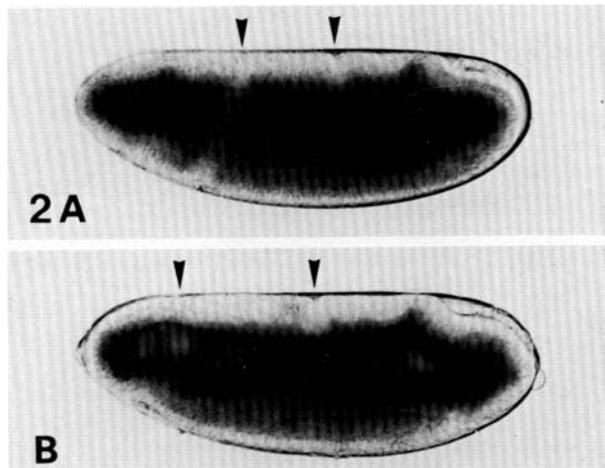


Fig. 2. Living embryos during the gastrulation, (A) unpricked, (B) pricked at the anterior tip during very early cleavage (experimental design A). Anterior left, ventral side bottom of the picture. The arrows point to the cephalic furrow (left) and posterior dorsal fold. In the embryo in B the cephalic furrow is not yet readily visible but appeared soon after the picture was taken.

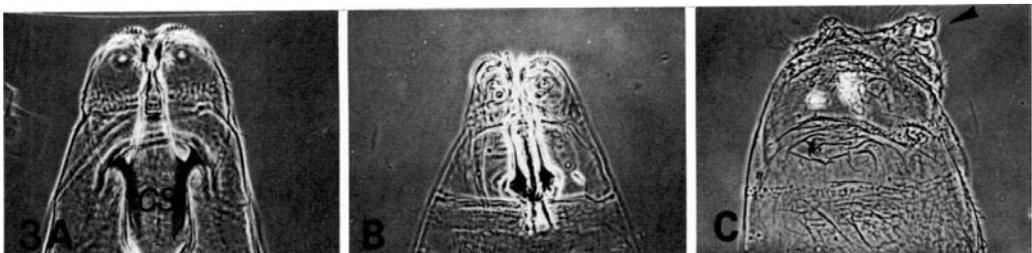


Fig. 3. The anterior region of embryos treated according to B (see Fig. 1). (A) Wild-type embryo. Note the sclerotized large cephalopharyngeal skeleton (cs). In the embryo in (B) it is much reduced. It is absent in the embryo in (C). Instead, some sensory cones (arrow) and a tuft are found as anterior-terminal structures in (C). Phase-contrast photographs.

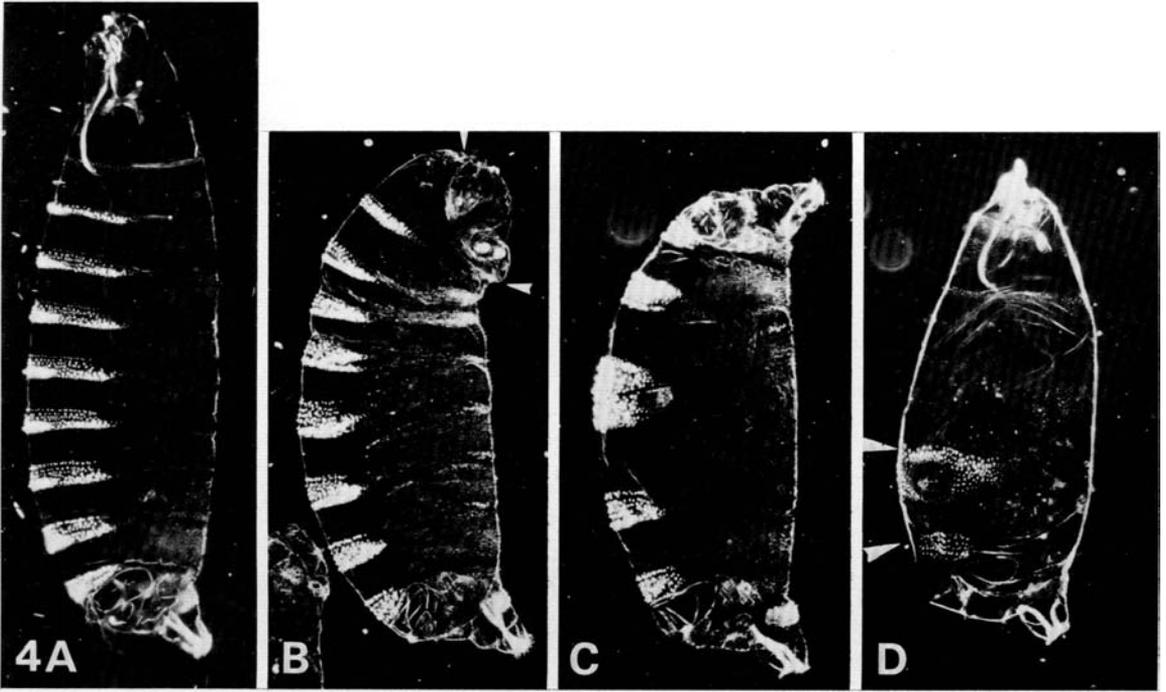


Fig. 4. Embryos treated according to experimental design D (B,C) or experiment E (D). (A) shows a normal untreated larva. The embryo in (B) has a complete abdomen and one thoracic segment. Anteriorly, a complete telson, including analplates, Filzkörper, spiracles and tuft, is duplicated (arrows). The embryo in (C) shows a perfect mirror-image duplication of the hindend including the telson and three abdominal segments. In (D) an embryo is shown that has been pricked at the posterior pole. Almost the entire abdomen, except for the first and last abdominal segments (arrows), is lacking while head, thorax and telson are normal.

Experiment C

Transplantation of cytoplasm from the middle of the egg has no consistent deleterious effect on development. The few cases with small heads obtained may have been caused by some leakage.

Experiment D

Simultaneous removal of anterior plasm and transplantation of posterior plasm anteriorly strongly enhance the effect of either treatment alone. Moreover, a new phenotype appears and double abdomens are formed in which the development of anterior structures like head, thorax and anterior abdomen is completely suppressed and instead a posterior end in reversed polarity is formed in the anterior half of the egg. The double abdomen embryos may show perfect mirror-image symmetry (Fig. 4C); more frequently however, the anterior reversed portion is smaller than the one in normal orientation. Often, only telson structures are duplicated anteriorly without apparent plane of mirror symmetry (Fig. 4B). The relative frequencies of these phenotypes (Table 1) depends not only on the age of

the recipient embryos, but also on the amount of the transplanted pole plasm. Transplantation of one pole plasm per recipient embryo causes less than 30 % of the embryos to develop mirror-image duplications, whereas this phenotype is produced in almost all embryos (19/21) that have received pole plasms from two donor embryos.

Experiment E

Removal of cytoplasm from the posterior tip of the embryos often causes segmentation defects in the abdomen, while the structures of the posterior-terminal telson are usually not affected (Table 2, Fig. 4). Predominantly central segments rather than the posterior-terminal ones are lacking (data not shown). Observations on early development of these embryos reveal that the number of pole cells is often reduced. Otherwise the embryos have a normal appearance, particularly the position of the dorsal folds and the cephalic furrow are unaffected. Thus the partial removal of pole plasm has a rather specific effect on the development of the abdominal region which is not immediately adjacent to the site of puncture.

Experiment F

Removal of cytoplasm from the prospective abdominal region, in the majority of cases, does not affect segmentation. Most of the embryos hatch as morphologically normal larvae regardless of whether the removal was done midventrally or from more lateral positions.

CONCLUSIONS

These experiments give evidence for factors with long-range organizing properties localized at the anterior and posterior poles of the *Drosophila* egg. Treatment at the anterior pole, experiments A and B, be it removal of anterior material or addition of posterior material, has qualitatively the same effect on embryonic development: the posterior pattern is expanded at the expense of anterior-terminal structures. In the extreme case, posterior-terminal structures appear at the anterior tip of the egg, suggesting that factors are localized in the anterior

Table 2. *Abdominal defects caused by removal of pole plasm*

Experimental design*	Number of surviving embryos	Embryos with the patterns (% of surviving embryos)			
		abdominal segments lacking			
		normal	2 or less	more than 2	telson defective
E	76	67	15	18	5
F†	68	90	10	0	1

* See Fig. 1.

† Embryos were pricked at 30 % egg length either midventrally or laterally.

plasm that are required for anterior development and inhibit posterior development. Partial removal of these factors has a long-range effect on the pattern along the entire anteroposterior egg axis as seen in the shift of the folds occurring at gastrulation towards more anterior positions. The same change in phenotype induced by transplanted posterior pole plasm suggests that there are factors localized at the posterior of the egg that have functions antagonistic to those of the anterior factors. The induction of a hindend including inverted abdominal segments at the anterior pole arranged in perfect mirror-image symmetry to the normal hindend requires the synergistic effects of depletion of anterior factors and supply of posterior factors and cannot, as in *Smittia*, be created by anterior pricking alone. In our transplantation experiments, we have not examined the recipient embryos for pole cell formation at the anterior pole, because this test, involving a second transplantation, would have excluded a careful analysis of the somatic pattern. It appears likely, however, that those embryos with pole cells at the anterior pole reported by Illmensee & Mahowald (1974) also had posterior somatic structures induced at the same site.

The removal of posterior pole plasm (experiment E) leads to pattern defects that are qualitatively different from those described for anterior plasm. Rather than a coordinated shift in developmental fate the segmentation in a specific sharply defined region, the abdomen, is impaired. The formation of the telson, the posterior-terminal somatic structure, is in general not affected. The independence of telson formation on posterior factors was already suggested by the results of experiment A in which telson duplications could be created by depletion of anterior factors alone.

The very specific and distinct phenotypes produced by these simple experiments have their counterparts in the phenotypes of several maternal effect loci. Embryos with reduced heads are produced by females mutant for alleles at the loci *swallow* (formerly called *fs(1) 1502*, Gans, Audit & Masson, 1975; Stephenson, personal communication; Nüsslein-Volhard, Wieschaus & Jürgens, 1982) or *exuperantia* (Schüpbach & Wieschaus, 1986). Duplication of the telson only is the extreme phenotype of the locus *bicoid* (Frohnhofer & Nüsslein-Volhard, in preparation). *Bicaudal* females produce the entire spectrum of posteriorization observed in our pricking and transplantation experiments (Nüsslein-Volhard, 1977, 1979). The phenotypes observed after removal of posterior pole plasm are rather accurately mimicked in mutants of the 'grandchildless-knirps' class of maternal effect genes (Schüpbach & Wieschaus, 1986). Examples are the loci *tudor* (Boswell & Mahowald, 1985) and *oskar* (Lehmann & Nüsslein-Volhard, 1986). Mutant embryos lack a distinct pole plasm including polar granules. No pole cells are formed and the segmentation of the abdomen is severely affected (*tudor*) or completely abolished (strong *oskar* alleles).

The capacities of *Drosophila* pole plasm to ectopically induce posterior development are comparable to the symbiont ball translocations in *Euscelis*. Induction of new structures in both animals is associated with long-range effects that alter the fate map along the entire anteroposterior axis. In *Smittia*, rather than a continuous

spectrum of phenotypes with segments shifted coordinately, experimentally only a few discrete pattern alterations can be induced. Especially after centrifugation, phenotypes as different as double heads and double abdomens, or normal embryos and inverted 'normals' may arise simultaneously in the same experiment (Kalthoff, Rau & Edmond, 1982). This feature strongly suggests that autocatalytic mechanisms are involved in establishing anteriority or posteriority for either pole, with random initial advantages deciding the direction of the switch in the disoriented centrifuged egg.

The establishment of morphogenetic centres and long-range effects emanating from them may be regarded as two essential features of the pattern-forming system in insects. Sander (1960) proposed a double gradient hypothesis to describe his results on *Euscelis*, assuming that influences from both poles act together to specify the pattern at each point of the germband. Meinhardt (1977) using a mathematical model based on autocatalysis and lateral inhibition was able to simulate the same data postulating a single gradient only, with the highpoint at the posterior pole. He could also mimic the dynamics of double abdomen formation in *Smittia*; however it was not possible to explain the double heads with his model based on a single posterior gradient. Unfortunately with two gradients interacting with each other and both acting on the specification of the cells, the system becomes quite complicated. Moreover, the relative importance of the two terminal influences on the specification of cells may differ considerably among species, e.g. the posterior activity may not be needed to form heads in chironomid midges, but it is needed in dragon flies (Seidel, 1929). In insects with short germband it is hard to see how embryonic specification is related at all to any spatial organization of the egg, since in these eggs germbands can be induced artificially at arbitrary points (reviewed by Sander, 1976). It may be impossible to understand such a flexible system until it is analysed at the molecular level. However combining transplantation as a functional assay with the outstanding genetics and molecular biology of *Drosophila* should soon lead to the identification of the molecules involved in organizing the anteroposterior pattern of this insect.

We thank Gerd Jürgens, David Marcey and Pat O'Farrell for critical and stimulating discussion and Dominique Kaiser for typing the manuscript.

REFERENCES

- BOWNES, M. (1976). Defective development after puncturing the periplasm of nuclear multiplication stage *Drosophila* embryos. *Devl Biol.* **51**, 146–151.
- BOWNES, M. & KALTHOFF, K. (1974). Embryonic defects in *Drosophila* eggs after partial UV irradiation at different wavelengths. *J. Embryol. exp. Morph.* **31**, 329–345.
- BOWNES, M. & SANG, J. H. (1974). Experimental manipulations of early *Drosophila* embryos. I. Adult and embryonic defects resulting from microcautery at nuclear multiplication and blastoderm stages. *J. Embryol. exp. Morph.* **32**, 253–272.
- BOSWELL, R. E. & MAHOWALD, A. P. (1985). *tudor*, a gene required for assembly of the germ plasm in *Drosophila melanogaster*. *Cell* **43**, 97–104.
- BULL, A. L. (1966). Bicaudal, a genetic factor which affects the polarity of the embryo in *Drosophila melanogaster*. *J. exp. Zool.* **161**, 221–242.

- GANS, M., AUDIT, C. & MASSON, M. (1975). Isolation and characterization of sex linked female sterile mutants in *Drosophila melanogaster*. *Genetics* **81**, 683–704.
- HERTH, W. & SANDER, K. (1973). Mode and timing of body pattern formation (regionalization) in the early embryonic development of cyclorrhaphic dipterans (*Protosphormia*, *Drosophila*). *Wilhelm Roux Arch. EntwMech. Org.* **172**, 1–27.
- ILLMENSEE, K. & MAHOWALD, A. P. (1974). Transplantation of posterior polar plasm in *Drosophila*. Induction of germ cells at the anterior pole of the egg. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1016–1020.
- JUNG, E. (1966). Untersuchungen am Ei des Speisebohnenkäfers *Bruchidius obtectus* Say (Coleoptera). *Wilhelm Roux Arch. EntwMech. Org.* **157**, 320–392.
- KALTHOFF, K. (1979). Analysis of a morphogenetic determinant in an insect embryo (*Smittia* spec. Chironomidae, Diptera). *Symp. Soc. devl Biol.* **37**, 97–126.
- KALTHOFF, K., RAU, K. & EDMOND, J. C. (1982). Modifying effects of ultraviolet irradiation on the development of abnormal body patterns in centrifuged insect embryos (*Smittia* sp., Chironomidae, Diptera). *Devl Biol.* **91**, 413–422.
- KALTHOFF, K. & SANDER, K. (1968). Der Entwicklungsgang der Mißbildung "Doppelabdomen" im partiell UV-bestrahlten Ei von *Smittia parthenogenetica* (Diptera, Chironomidae). *Wilhelm Roux Arch. EntwMech. Org.* **168**, 129–146.
- KANDLER-SINGER, I. & KALTHOFF, K. (1976). RNase sensitivity of an anterior morphogenetic determinant in an insect egg (*Smittia* sp., Chironomidae, Diptera). *Proc. natn. Acad. Sci. U.S.A.* **73**, 3739–3743.
- LEHMANN, R., JIMÉNEZ, F., DIETRICH, U. & CAMPOS-ORTEGA, J. A. (1983). On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. *Wilhelm Roux' Arch. devl Biol.* **192**, 62–74.
- LEHMANN, R. & NÜSSLEIN-VOLHARD, C. (1986). The maternal gene *oskar* in *Drosophila*: *oskar* dependent activity is localized in the posterior pole plasm and required for pole cell formation, abdominal segmentation and embryonic polarity. *Cell* (in press).
- LOHS-SCHARDIN, M., CREMER, C. & NÜSSLEIN-VOLHARD, C. (1979). A fate map for the larval epidermis of *Drosophila melanogaster*: localised cuticle defects following irradiation of the blastoderm with an UV-laser microbeam. *Devl Biol.* **73**, 239–255.
- MAHOWALD, A. P. (1962). Fine structure of pole cells and polar granules in *Drosophila melanogaster*. *J. exp. Zool.* **151**, 201–215.
- MEINHARDT, H. (1977). A model for pattern formation in insect embryogenesis. *J. Cell Sci.* **23**, 117–139.
- NÜSSLEIN-VOLHARD, C. (1977). Genetic analysis of pattern formation in the embryo of *Drosophila melanogaster*. *Wilhelm Roux' Arch. devl Biol.* **183**, 249–268.
- NÜSSLEIN-VOLHARD, C. (1979). Maternal effect mutations that alter the spatial coordinates of the egg. *Symp. Soc. devl Biol.* **37**, 185–211.
- NÜSSLEIN-VOLHARD, C., WIESCHAUS, E. & JÜRGENS, G. (1982). Segmentierung bei *Drosophila* – eine genetische Analyse. *Verh. dt. zool. Ges.* **1982**, 91–104.
- SANDER, K. (1959). Analyse des ooplasmatischen Reaktions-systems von *Euscelis plebejus* Fall. (Cicadina) durch Isolieren und Kombinieren von Keimteilen. I. Mitteilungen. *Wilhelm Roux Arch. EntwMech. Org.* **151**, 430–497.
- SANDER, K. (1960). Analyse des ooplasmatischen Reaktions-systems von *Euscelis plebejus* Fall. (Cicadina) durch Isolieren und Kombinieren von Keimteilen. II. Die Differenzierungsleistung nach Verlagern von Hinterpolmaterial. *Wilhelm Roux Arch. EntwMech. Org.* **151**, 660–707.
- SANDER, K. (1976). Specification of the basic body pattern in insect embryogenesis. *Adv. Insect Physiol.* **12**, 125–238.
- SCHMIDT, O., ZISSLER, D., SANDER, K. & KALTHOFF, K. (1975). Switch in pattern formation after puncturing the anterior pole of *Smittia* eggs (chironomidae, diptera). *Devl Biol.* **46**, 216–221.
- SCHUBIGER, G., MOSELEY, R. C. & WOOD, W. (1977). Interaction of different egg parts in determination of various body regions in *Drosophila melanogaster*. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2050–2053.
- SCHÜPBACH, T. & WIESCHAUS, E. (1986). Maternal-effect mutations altering the antero-posterior pattern of the *Drosophila* embryo. *Wilhelm Roux' Arch. devl Biol.* (in press).

- SEIDEL, F. (1929). Untersuchungen über das Bildungsprinzip der Keimanlage im Ei der Libelle *Platycnemis pennipes* I–V. *Wilhelm Roux Arch. EntwMech. Org.* **119**, 322–440.
- VAN DER MEER, J. M. & MIYAMOTO, D. M. (1984). The specification of metameric order in the insect *Callosobruchus maculatus* Fabr. (Coleoptera). II. The effects of temporary constriction on segment number. *Wilhelm Roux' Arch. devl Biol.* **193**, 326–338.
- YAJIMA, H. (1960). Studies on embryonic determination of the harlequin-fly, *Chironomus dorsalis*. I. Effects of centrifugation and of its combination with constriction and puncturing. *J. Embryol. exp. Morph.* **8**, 198–215.
- YAJIMA, H. (1964). Studies on embryonic determination of the harlequin-fly, *Chironomus dorsalis*. II. Effects of partial irradiation of the egg by ultraviolet light. *J. Embryol. exp. Morph.* **12**, 89–100.

