# The evolution of insect patterning mechanisms: a survey of progress and problems in comparative molecular embryology

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## SUMMARY

This report surveys data and interpretations presented by speakers in the Arthropod Session of the 1994 BSDB Spring Symposium. After a short review of phylogenetical aspects in premolecular insect embryology, the following topics are discussed: the ancestral germ type of pterygote insects, correlations between oogenesis and embryonic pattern formation, the universality or otherwise of *bicoid* as the anterior morphogen, novel functions in the insect Hox complex, the formal asymmetry between evolution and decay of complex gene networks, novel regulatory interac-tions as the main cause of evolutive changes, the repeated activity of conserved gene networks in successive steps of ontogenesis and strategies for future research. Interspersed are some unpublished data on oogenesis and pattern formation in lower dipterans, and their possible evolution-ary implications.

Key words: insect, patterning, germ type, *bicoid*, lower dipterans, oogenesis, pattern formation, Hox

## INTRODUCTION

Research into embryogenesis was tremendously stimulated by Charles Darwin's 'Origin of Species', and especially by his view that the study of ontogenesis might offer the most convincing testimony of evolution. His expectations were based on the mor-phology of embryonic stages, but many articles in the present volume show that the study of ontogenesis with molecular methods holds even more promise. Not only can it yield a wealth of novel information on the probable course of evolution but it can also reveal molecular and developmental changes that have led – and may still lead – to overt morphological change.

The molecular approach to evolution has advanced farther (as yet) in the insects than in any other group, because insects combine a sufficient degree of complexity and variation with many technical advantages – a combination that has made *Drosophila* the organism of choice for the pioneers of classical as well as developmental genetics, and now qualifies this species to serve as the reference system for comparative molecular embryology.

Below I shall give a brief outline of past thoughts on the evo-lutionary aspect of comparative insect embryology, and then try and highlight some of the progress and the problems that have become evident during the session on arthropod development in this BSDB symposium. The individual contributions published in the present volume will be quoted here by authors' names only, without the year, except when they are first mentioned.

## THE PHYLOGENETICAL ASPECT IN PREMOLECULAR INSECT EMBRYOLOGY

Even before Darwin's 'Origin', the (very few) authors writing on insect embryogenesis had considered problems akin to phylogeny, in that they searched for unifying concepts under which to subsume the course of embryogenesis in both insects and vertebrates (reviewed in Nübler-Jung and Arendt, 1994). As in comparative anatomy, their method was to search for homologies. While the origins of these homologies were usually left open, some interspersed statements have a ring of evolutionary thinking. Albert Kölliker for instance wrote in 1842 that "as we all know, in higher animals an organ at the onset and during its development reflects the form with which it is endowed in lower animals". Such statements probably betray a widespread feeling in the years between Lamarck and Darwin that life (contrary to Lamarck's concept) was created only once and that therefore "only the external form of the appearances of life is subject to continuous change, tied to the external conditions which either favour or prevent its devel-opment..." (J. C. Pander 1821, as quoted by Bäumer-Schleinkofer, 1993; translation by the present author).

Almost a century later, the comparison of embryogenesis between different insect species – now of course firmly embedded in concepts of phylogenesis – reached its first climax in the doctoral dissertation of Friedrich Seidel (1924). At that time, Seidel relied exclusively on morphological data, but his interpretations expressly implied profound differences between the patterning mechanisms involved; indeed he clas-sified insect embryos in a series ranging from the 'non-deter-minative' to the strictly 'determinative' developmental type. In subsequent experimental work (reviewed e.g. in Counce and Waddington, 1972; Sander, 1976), Seidel and his students demonstrated that corresponding functional differences exist. They thereby abolished the view that the insect egg cell embodies a complex mosaic of localized maternal determi-

nants (an accepted wisdom hailing largely from Hegner's work on germ cell determination, reviewed in Sander, 1984). Their assembled descriptive and experimental data enabled Krause (1939a,b) to propose a graded series of 'insect egg types' which were primarily intended to provide a conceptual frame for physiological investigations. But this series, according to Krause (1939a) "will also please the taxonomist... in that the eggs as types can be arranged in correspondence with the adults" – and thus, implicitly, with the phylogenetic tree.

Krause's terms for the main types or modes of early insect embryogenesis – short, half-long (now called intermediate) and long germ – are still with us and pervade the relevant contributions in this volume. It may therefore be worthwhile to define them in his own words (translated from Krause, 1939b by the present author). "The 'short germ' mainly represents the head region (*Tachycines*), the 'long germ' maintains the natural proportions of the body regions of the larva (*Apis*). Therefore short germ, half-long germ and long germ differ by the number of presumptive segments within the segment formation zone", the posterior blastema which would generate these segments successively by proliferation.

Some 30 years later, at the eve of the 'molecular revolution' of insect embryology to be rung in by the herculean genetic studies of Lewis (1978) and Nüsslein-Volhard and Wieschaus (1980), embryogenesis in various groups of the Articulata was exploited by Anderson as a guide to establishing phylogenetic relationships (Anderson, 1973). Soon afterwards, the evolution of patterning mechanisms was briefly invoked by the present writer when the increasing role of anterior polar determinants during insect phylogenesis was recognized (Sander, 1976), and was treated more extensively at a subsqent BSDB meeting (Sander, 1983).

### THE ANCESTRAL MODE OF EARLY EMBRYOGENESIS IN PTERYGOTE INSECTS – SHORT-GERM OR INTERMEDIATE TYPE?

On this question, opinion was and still is divided. Patel (1994) implies the possibility that short-germ development is ancestral. In favour of this view speaks the sequential budding of segments that is reminiscent of development in marine annelids. Its shortcoming is that orthopterans do not start segmenting (and expressing *engrailed*, see Patel) right behind the head lobes but rather in the thorax; however, this might be a secondary specialization, to be viewed as a corollary of the fact that the largest limb buds form in that region (Fleig, 1990). Tautz et al. (1994), in contrast, like Anderson (1972, 1973) favour the intermediate mode as ancestral, drawing on the fact that this mode apparently prevails in the Odonata (damsel- and dragonflies), considered by many to be the most primitive living pterygotes.

The short-germ hypothesis implies that the pair-rule level of patterning (Nüsslein-Volhard and Wieschaus, 1980) evolved within the Pterygota – that is unless pair-rule patterning should yet be found in orthopterans. The concept of Anderson (1972) and Tautz et al., however, would mean that patterning in the anterior body half might from the beginning have employed a pair-rule mechanism (perhaps of diplopod origin? cf. Sander, 1988), which was subsequently lost in the lineages leading to short-germ insects. This view might prevail if pair-rule pat-

terning were indeed demonstrated in the anterior regions of odonate embryos, and if everyone agreed that the Odonata are really closer to the ancestral pterygote than the orthopterans or other primitive forms, among them the ephemerids and stone flies, of which the latter clearly follow the short-germ mode (Miller, 1939). In favour of an odonate-like ancestor is the fact that only in the Odonata do the yolk cells yield part of the midgut wall (Ando, 1962; see also Anderson, 1972) whereas midgut development in all other pterygote insects seems highly derived.

### GERM TYPE CLASSIFICATION AT THE MOLECULAR LEVEL OF RESOLUTION?

The controversy over the ancestral germ type may resolve in yet another and hitherto unsuspected way, namely by a reclassification of germ types using molecular in addition to morphological criteria. The need for a more sophisticated classification is apparent from Patel's demonstration that the short-germ mode may exhibit considerable molecular differences, and from the observations of Kraft and Jäckle (quoted by Tautz et al.) that the seemingly short-germ tobacco hawkmoth expresses in its blastoderm the full number of stripes of some pair-rule and segment polarity genes. Conversely, the honeybee, which morphologically represents the prototype of long-germ development (see quotation from Krause above), was shown to generate its abdominal *engrailed* stripes in an anteroposterior sequence reminiscent of intermediate- or even short-germ development (Fleig, 1990). These and other discrepancies, some of which were noted already by Krause (1939b), call for a revision, and Patel's approach of exploiting the temporal relationship between gastrulation and molecular segment specification may be a first step towards a more satisfactory classification.

### OOGENESIS IN RELATION TO GERM TYPES AND PATTERN FORMATION

The first generalized correlations between modes of oogenesis and types of early embryogenesis were established by Bier (1970) who linked short-germ development with panoistic, and long-germ development with meroistic-polytroph oogenesis (reviewed in Sander, 1976). However, comparing this generalization with the examples listed by Krause (1939b) for the different germ types will reveal exceptions, for instance the apparent long-germ development in cockroaches (where oogenesis is panoistic) or the apparent short-germ development in some – meroistic – beetles. May be these inconsistencies will disappear with a better classification of germ types (see the previous section) but it is worthwhile to ask, with Tautz et al., whether long-germ development really requires molecular and cellular innovation in oogenesis, specifically the addition of nurse cells. Perhaps the cytoskeletal mechanisms known from panoistic development (and general cell biology) might suffice for determinant localization, as suggested by Tautz et al., but the fact is that in *Drosophila* the nurse cells enforce an anterior course of development on the oocyte pole(s) to which they are attached by ring canals (Bohrmann and Sander, 1987). The related question whether *bcd* is a general determinant of

'anteriorness' throughout the pterygota will be discussed below – after a look at another maternal patterning component, namely the terminal class genes which Tautz et al. suggest to be ancestral.

The expression of the maternal terminal gene *torso-like* occurs in the polar cells of the follicular epithelium (Martin et al., 1994). Of these, the anterior group are known in *Drosophila* as the border cells once they have migrated through the nurse cell cluster to reach the anterior oocyte pole. The border cells produce, during late oogenesis, the micropylar canal and part of the surrounding egg envelopes (reviewed in Spradling, 1993). Surprisingly, in lower dipterans, these cells do not migrate but stay anterior to the nurse cell(s) and assemble the micropyle in this ectopic position. The micropyle then comes to touch the anterior oocyte pole only when the nurse cell(s) have shrivelled away. This holds not only for *Bradysia* (syn. *Sciara*) (Wenzel et al., 1990), where the single nurse cell might leave no space for migration, but also for psychodids (moth midges) which, like *Drosophila*, have 15 nurse cells. These findings (our unpublished results) might gain wider evolutionary interest when viewed together with two other pieces of information: (1) the *torso-like* signal is believed to reside temporarily in the vitelline envelope (see St Johnston and Nüsslein-Volhard, 1992), and (2) in many insect species (e.g. the stick insects reviewed in Sander, 1983) the micropyle is situated in the posterior egg half and the head lobes form next to it while the more anterior egg parts are probably dispensable for pattern formation. It might be worthwhile testing, once suitable molecular probes become available, whether in such species the micropyle-forming follicle cells also emit a 'polar' signal which in turn might define the anterior limits, and maybe axial polarity, of the future germ band.

## IS BICOID UNIVERSAL?

The *bicoid* protein has provided the first and as yet best-analyzed molecular example of a maternally specified morphogenetic gradient (reviewed in St Johnston and Nüsslein-Volhard, 1992). Notwithstanding these epochal merits, it may be legitimate to question its universality. Akam et al. (1994) point out that *bcd* is one of a group of rapidly evolving nonhomeotic genes within the Antennapedia complex of insects (but not found in any other taxon), and that *bcd* homologs have been recognized so far only in fruit-, house- and blowflies. Even within these groups, some differences are evident, especially among the blowflies (Schröder and Sander, 1993) where *Calliphora* differs from the other species in both mRNA localization and the fact that its anterior ooplasm has so far failed to rescue *Drosophila bicoid* embryos.

Akam et al. suggest that a Hox class 3 gene recently isolated from the locust *Schistocerca* may share a 'common ancestor' with both *zen* and *bicoid*, which would imply that the *bicoid* function arose late in insect evolution. Assuming that lower dipterans reflect the ancestral dipteran stock, they might provide some relevant information. As mentioned earlier (Sander, 1988), centrifugation can easily and quantitatively induce the eggs of lower dipterans to form mirror-image patterns of the double cephalon or double abdomen type, whereas this is very hard to achieve in wild-type *Drosopila* embryos. However, centrifuged eggs from *bcd* mutant flies are quite prone to double abdomen formation; the yield is inversely correlated to the strength of the *bcd* allele(s) used, and with the strongest alleles (e.g. E1) can approach 100% (Schröder, 1992). There may be many explanations for this, but in our context the most tempting would be that lower dipterans develop without *bicoid*, perhaps using *hunchback* as the anterior determinant as envisioned by St Johnston and Nüsslein-Volhard (1992). In line with this, attempts to isolate from the lower dipteran *Psychoda* a *bcd* homolog have failed so far (R. Schröder, unpublished result). Another potentially relevant difference between lower and higher dipterans was observed in mirror-image duplication patterns. In *Drosophila*, symmetrical double abdomens comprise less segments than in lower diperans (Percy et al., 1986, and our unpublished data), while *Drosophila* double cephalons contain significantly more segments than their counterparts in lower dipterans (our unpublished results). These findings signal that the longitudinal patterning mechanisms of lower dipterans differ considerably from those of *Drosophila* – perhaps owing to the absence of *bcd* and a concomitant shift of gap gene expression domains?

## CONSERVED GENES AND NOVEL FUNCTIONS IN THE HOX COMPLEX

The extreme conservation of the homeotic genes in the Hox complex(es) enabled Carroll (1994) and Akam et al. to test a famous proposal concerning a gene of this class. Lewis (1978) had suggested that the *Ubx* gene is an evolutionary novelty of the dipterans which evolved with (or rather for) the suppression of wings in the metathorax. However, Akam et al. show that *Ubx* and all other homeotic *Drosophila* Hox genes have their homologs in crustaceans, even in species with almost uniform trunk segments. Moreover, butterflies according to Carroll express *Ubx* in their winged metathorax. Both findings mean that the *Ubx* protein as such must be much older than its apparent function in *Drosophila*; what has changed during evolution is obviously the network of target genes regulated by the *Ubx* protein (see below). Interestingly, the regulation of this gene itself seems to differ even within the holometabolous insects. For instance, Carroll has shown that in the abdominal segments of the silkmoth both *Ubx* and *abd A* are locally down-regulated in the abdominal cell patches that subsequently give rise to the proleg buds.

## ASYMMETRY BETWEEN EVOLUTION AND DECAY OF COMPLEX SYSTEMS

The extreme conservation of the homeotic genes certainly has to do with their fundamental regulatory role in establishing the phylotypic body plan, which should require very complex networks. Akam et al. by their comparative analysis of the non-homeotic Hox gene *ftz*, now provide a quantitative measure for this conservation: *ftz* evolves about ten times faster than the homeotics in the same complex. The *ftz* gene may be less subject to stabilizing selection because, as indicated by the homeobox sequences, the interaction of its protein with other regulatory proteins may be less complex.

The new data on regulatory networks are highlighting an old but sometimes forgotten insight concerning acquisition and

loss, respectively, of complex characters. Regulatory networks can evolve by successive accumulation of many changes in the target genes. However, abolition of characters generated by this tedious process may require just the mutative loss of function in a single regulatory gene. This asymmetry must be borne in mind when it comes to deciding whether a given, seemingly ancestral trait, for instance homonomous segmentation (Akam et al.) or wings in a dipteran metathorax (Carroll), is really primitive or not.

## MORPHOLOGICAL INNOVATION FOLLOWS FROM NOVEL REGULATORY INTERACTIONS RATHER THAN NEW PROTEINS

To quote Carroll, "the chemical evolution of of animals has not been nearly as great as their morphological evolution". This insight, too, is not quite new, but it can now be supplemented with a wealth of hard molecular data. Instances are the sophisticated regulation mechanisms for and by *Ubx*, evolved perhaps by changes at the enhancer level, which enable a single protein to specify the characters of several abdominal segments (Akam et al.), or the recruiting of genes that serve to specify cell fates in the central nervous system (e.g. *ftz* and *eve*) for pair-rule functions in the segmentation cascade (Patel). The most striking evidence, however, comes from the repeated 'deployment' of certain genes and regulatory networks in the course of a single ontogenesis.

## REPEATED ACTIVITY OF CONSERVED REGULATORY NETWORKS AND GENES

The classical example for this is the expression of certain vertebrate Hox complexes that specify cell fates first along the body axis and thereafter in the appendages (see Duboule, 1994). Among insects, wing development in butterflies (Carroll; Nijhout, 1994) has now provided another instance. The genes which in *Drosophila* play key roles in specifying the spatial organization of the wing disc have homologs with similar functions in the butterfly *Precis coenia*. This reflects a common overall organization of wing morphogenesis in both species. However, in the butterfly, these genes later on are transcribed again, this time in each of the 'wing cells' (demarcated by the wing veins) where they apparently are involved in specifying the beautiful patterns of coloured scales. The most striking pattern element, the eye spot, is organized from its center by yet another 're-deployed' gene, namely *Distalless*, which earlier on is expressed (and required) in the prospective distal parts of embryonic appendage buds and in the imaginal discs of late larval stages.

## STRATEGIES FOR FUTURE RESEARCH IN COMPARATIVE MOLECULAR EMBRYOLOGY

The strategies for future research mentioned implicitly or expressly in the relevant contributions to this symposium fall in two classes: strategies that are confined to the concepts and probes provided by molecular research on *Drosophila* development, and others that propose to evade the limitations that the prevalent "*Drosophila*-centric view" (Carroll) imposes on our prospects. As Patel points out, the highly successful exercise of identifying homologs of *Drosophila* segmentation genes in other insects will fail to identify any patterning mechanisms that *Drosophila* might be lacking. He therefore proposes to seek out additional organisms amenable to genetic analysis. But remembering the long history of *Drosophila* research and the concurrent input of both intellect and money, this may not be easily achieved. Those eager to try might be well advised to study the list of requirements that Carroll has drawn up for his rather less ambitious approach. As a counterpart to *Drosophila* for genetical analysis, this writer would recommend saprophagous lower dipterans, e.g. the psychodids and scatopsids (see Schmidt-Ott et al., 1994). They are easily mass-reared with short generation times, have beautiful embryos and, last but not least, differ surprisingly from *Drosophila* both developmentally (see above) and, if the first relevant data (Sommer et al., 1992) are representative, also at the molecular level.

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