

The evolution of developmental gene networks: lessons from comparative studies on holometabolous insects

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Recent comparative studies have revealed significant differences in the developmental gene networks operating in three holometabolous insects: the beetle *Tribolium castaneum*, the parasitic wasp *Nasonia vitripennis* and the fruitfly *Drosophila melanogaster*. I discuss these differences in relation to divergent and convergent changes in cellular embryology. I speculate on how segmentation gene networks have evolved to operate in divergent embryological contexts, and highlight the role that co-option might have played in this process. I argue that insects represent an important example of how diversification in life-history strategies between lineages can lead to divergence in the genetic and cellular mechanisms controlling the development of homologous adult structures.

Keywords: Insecta; sequential segmentation; long germ development; convergent evolution; co-option of genes

1. INTRODUCTION

Arthropods are defined by a segmented body plan consisting of a series of antero-posteriorly arrayed segmental units with associated jointed appendages. The insects are traditionally viewed as one of the four major monophyletic arthropod groups, the other three being crustaceans, myriapods and chelicerates (Brusca & Brusca 2003). However, recent molecular phylogenies suggest that crustaceans are paraphyletic with respect to the insects; i.e. insects could reasonably be regarded as a monophyletic clade of terrestrial crustaceans (Carapelli *et al.* 2007). The holometabolous insects undergo complete metamorphosis from larva to adult via a pupal stage (Brusca & Brusca 2003). This is considered a derived life-history trait that arose only once during insect evolution (Brusca & Brusca 2003); see figure 1. Other insect species have retained the ancestral condition of undergoing metamorphosis from larva to adult through a series of intermediate nymphal stages (the hemimetabolous insects; figure 1). The vast majority of holometabolous insects belong to four speciose orders: the Diptera (two-winged flies), the Lepidoptera (butterflies and moths), the Coleoptera (beetles) and the Hymenoptera (ants, bees, wasps etc.). We currently have a better understanding of the developmental genetic network underlying segmentation in a member of the Diptera—the fruitfly *Drosophila melanogaster*—than for any other insect, or indeed arthropod (Lawrence 1992); see figure 2. However, a representative of the Coleoptera, the beetle *Tribolium castaneum*, and a representative of the Hymenoptera, the parasitic wasp *Nasonia vitripennis*, are rapidly being established as powerful model insect systems (Choe *et al.* 2006; Brent *et al.* 2007). Recent

studies have revealed significant differences in the segmentation gene networks operating in these insects when compared with each other and *D. melanogaster* (Schröder 2003; Bucher & Klingler 2004; Cerny *et al.* 2005; Choe *et al.* 2006; Lynch *et al.* 2006a,b; Olesnický *et al.* 2006; Brent *et al.* 2007; Choe & Brown 2007). In this paper, I review and discuss these differences in relation to the modes of cellular embryogenesis exhibited by these insects. Both *D. melanogaster* and *N. vitripennis* have evolved a rapid mode of development that required major changes in embryogenesis at the cellular level (Bull 1982; Lawrence 1992; Davis & Patel 2002). In contrast, *T. castaneum* has retained a more ancestral mode of cellular embryogenesis (Handel *et al.* 2000; Davis & Patel 2002). I speculate on how insect segmentation gene networks have evolved to operate in these divergent embryological contexts. A recent molecular phylogeny suggests that the rapid mode of cellular embryogenesis exhibited by *D. melanogaster* and *N. vitripennis* evolved convergently (Savard *et al.* 2006). I go on to ask whether convergent gene network changes might have underpinned these apparent parallel transitions in cellular embryology. First, however, I review the modes of cellular embryogenesis found within the insects, and discuss the role that life history has played in their evolution.

2. THE INFLUENCE OF LIFE-HISTORY STRATEGY ON INSECT EMBRYOGENESIS

(a) *An evolutionary biologist's view on development*

The principal aim of a developmental biologist is to work towards establishing a more complete picture of how the genetic information contained within an organism's genome is deployed over developmental time to transform a single cell into a functional multicellular organism. In contrast, the principal aim

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order	species	hemi- or holo-metabolous	extended syncytial blastoderm	sequential or long germ segmentation	anterior localization of maternal mRNAs
Orthoptera	<i>Schistocerca</i> sp. <i>Gryllus bimaculatus</i>	hemi	no	seq	?
Hemiptera	<i>Oncopeltus fasciatus</i>	hemi	?	seq	?
Hymenoptera	<i>Nasonia vitripennis</i>		yes	long	yes
	<i>Bracon hebetor</i>	holo	yes	long	?
	<i>Aphidius ervi</i>		no	seq	?
	<i>Apis mellifera</i>		yes	long*	?
Coleoptera	<i>Tribolium castaneum</i>	holo	yes	seq long*	yes ?
	<i>Callosobruchus maculatus</i>				
Lepidoptera	<i>Bombyx mori</i>	holo	no	seq*	?
	<i>Manduca sexta</i>		?	long*	
Diptera	<i>Drosophila melanogaster</i>	holo	yes	long	yes
	<i>Anopheles gambiae</i>				

Figure 1. A phylogeny of the insect species discussed in this review with embryological features mapped on. The relative relationships of the four holometabolous insect orders follow the study by Savard *et al.* (2006). Some insects do not fit comfortably into the categories 'sequential' or 'long germ' segmentation; for caveats in relation to the categorization of specific species (*) see Davis & Patel (2002). Character states have been left clear where there are uncertainties, i.e. when there is a lack of gene expression data and/or dye injection experiments to ascertain the existence of an extended syncytial blastoderm stage.

of many evolutionary developmental biologists is to identify—through comparative analysis of developmental data within a phylogenetic framework—the changes in developmental mechanisms that underpinned divergence in body architecture between lineages. Rather than thinking in terms of developmental time—with the rather arbitrary starting point of zygote or germ cell—evolutionary developmental biologists consider evolutionary time scales, and as such development is not viewed as a linear process, but rather as continuous developmental cycles undergoing constant modification in response to selection and drift.

Natural selection can act independently on distinct stages of an organism's developmental cycle. This is obvious when considering insects. There has clearly been divergence in segment form between insect species, particularly with respect to appendage morphology—compare, for example, the sucking mouthparts of the phytophagous milkweed bug *Oncopeltus fasciatus* (Hughes & Kaufman 2000) with the mandibles of some carnivorous beetles (Konuma & Chiba 2007). However, it is clear that, on the whole, the basic insect segmental unit has been conserved. In contrast, insect oocytes and early eggs exhibit significant morphological differences, a consequence of the numerous and diverse life-history strategies that have evolved within the insects.

(b) All eggs are different, but some eggs are more different than others

Evolutionary shifts in insect life-history strategies often correlate with changes in cellular modes of embryogenesis. This was dramatically illustrated in a study by Grbic & Strand (1998) on two parasitic wasps

belonging to the hymenopteran family *Braconidae*. *Bracon hebetor* is an ectoparasite that lays yolky eggs on the integument of moth larvae. In the lineage leading to *Aphidius ervi*, however, there has been a transition to an endoparasitic life history; *A. ervi* lays a single yolkless egg into the haemocoel of an aphid host. Grbic & Strand (1998) studied the cellular embryology of these insects and found significant differences. In the eggs of *B. hebetor*, the cellularization of early cleavage nuclei is delayed until after they form a blastoderm, and all segments develop more or less simultaneously. In contrast, in *A. ervi* eggs, complete cytokinesis (the formation of cell membranes) occurs from the fourth round of nuclear divisions onwards, the early embryo ruptures from the chorion within the host haemocoel, and segments form one by one in an anterior to posterior progression. One can only speculate on why the transition to an endoparasitic life history required such dramatic changes in cellular embryology, but it seems likely that they are associated with the transition from receiving nutrients in the form of maternal yolk to the use of nutrients available from the haemolymph of the unfortunate host.

Similarly dramatic cellular transitions in embryogenesis have occurred within non-parasitic insect lineages (for an in-depth review see Davis & Patel 2002). Although the precise ecological reasons remain speculative, it seems likely that in many cases these transitions occurred in response to selection for increases in the speed of embryogenesis. Here I discuss two specific cellular adaptations and how they might have facilitated the faster development of an insect segmented body plan.

(i) *The timing of cellularization*

In most insect species early nuclear divisions are superficial; the formation of cell membranes around early cleavage nuclei is delayed until they have migrated to the egg surface and formed the blastoderm. For example, dye injection experiments have demonstrated this to be the case in the locust *Schistocerca gregaria* (Ho *et al.* 1997); figure 1. However, this delay is particularly pronounced in some holometabolous insect lineages, creating an extended syncytial blastoderm stage: examples of such insects include *N. vitripennis* (Bull 1982), *T. castaneum* (Handel *et al.* 2000) and *D. melanogaster* (Lawrence 1992); figure 1. Within a syncytium, gradients of patterning molecules can form quickly across a field of nuclei via diffusion, without the need for complex intercellular signalling pathways.

(ii) *The allocation of cells to segments*

The temporal dynamics by which cells are allocated to segments varies across insect species. In insects exhibiting primitive modes of development, anterior segments are patterned through the subdivision of blastoderm nuclei/cells, while posterior segments are patterned sequentially after the blastoderm stage, within a posteriorly located cellular zone of extension. Examples of such insects include the hemimetabolous insects *Gryllus bimaculatus*, *Schistocerca* sp. and *O. fasciatus* and the holometabolous insect *T. castaneum* (see Davis & Patel 2002; figure 1). I shall refer to these as 'sequentially segmenting' insects. In many insect lineages there has been an increase in the number of anterior segments patterned through subdivision in the blastoderm (Davis & Patel 2002). For example, this has occurred in some coleopteran lineages (Patel *et al.* 1994). In many holometabolous insects this trend has reached its extreme, and all segments form through early subdivision of embryonic blastoderm nuclei. These insects are said to exhibit 'long germ' embryogenesis, since the embryonic germ rudiment typically occupies almost the entire egg length. Examples of such insects include *D. melanogaster* (Lawrence 1992) and *N. vitripennis* (Bull 1982); see below and figures 1 and 2.

3. MOLECULAR TRANSITIONS UNDERLYING THE EVOLUTION OF LONG GERM EMBRYOGENESIS

Both the fruitfly *D. melanogaster* and the parasitic wasp *N. vitripennis* exhibit long germ embryogenesis. During long germ embryogenesis in *D. melanogaster*, a cascade of transcription factors acts within a syncytium to divide the embryo into progressively smaller domains such that segments develop more or less simultaneously. The *D. melanogaster* segmentation gene cascade is briefly outlined in figure 2, but for a more thorough understanding the reader is referred to Lawrence (1992).

In order to identify the changes in gene networks that underpinned the evolution of long germ embryogenesis, a good understanding of the segmentation mechanisms operating in insects that have retained sequential segmentation is required. One such insect is the beetle *T. castaneum* (Handel *et al.* 2005). Recent studies on this holometabolous insect have revealed significant differences in the genetic circuitry underlying

segmentation when compared with *D. melanogaster* (Schröder 2003; Bucher & Klingler 2004; Bucher *et al.* 2005; Cerny *et al.* 2005; Choe *et al.* 2006; Choe & Brown 2007). Together, comparative studies on *T. castaneum*, *N. vitripennis* and *D. melanogaster* suggest that the evolution of long germ embryogenesis required distinct molecular transitions to occur in concert at either pole of the egg (Choe *et al.* 2006; Brent *et al.* 2007).

(a) *Molecular transitions at the anterior egg pole: the evolution of maternally encoded anterior patterning gradients*

In *T. castaneum*, head and thoracic segments are patterned through the subdivision of blastoderm nuclei located near the posterior egg pole; the anterior blastoderm forms extraembryonic tissue (Handel *et al.* 2000). In insects exhibiting long germ embryogenesis, however, head and thoracic segments are patterned further towards the anterior egg pole. It has been proposed that this spatial shift in anterior patterning required the evolution of an instructive anterior patterning gradient to complement the action of existing posterior determinants (Lynch *et al.* 2006a). The localization of maternal mRNAs to the anterior pole of the oocyte is observed in *T. castaneum*, *N. vitripennis* and *D. melanogaster* (Lawrence 1992; Bucher *et al.* 2005; Olesnický & Desplan 2007); figure 1. In *N. vitripennis* and *D. melanogaster*, diffusion of mRNA and/or translated protein from anterior and posterior sources of maternal mRNAs form largely non-overlapping, and opposing, instructive patterning gradients (Lawrence 1992; Lynch *et al.* 2006a; Olesnický *et al.* 2006; Brent *et al.* 2007); figure 2 and table 1. In *T. castaneum*, on the contrary, neither of the anteriorly localized mRNAs identified to date play a significant role in anterior-posterior patterning (Bucher *et al.* 2005), and the maternal mRNAs of two genes known to be important anterior determinants in *T. castaneum*—*hunchback* and *orthodenticle-1*—are initially distributed uniformly in the egg (Wolff *et al.* 1995; Schröder 2003); see table 1. It is possible that an anteriorly localized maternal mRNA, whose protein product diffuses to form an instructive patterning gradient, exists, but has been overlooked, in *T. castaneum*. However, it is tempting to speculate that there is an association between the retention of sequential segmentation in *T. castaneum*, and the lack of an instructive anterior patterning gradient. It will be interesting to determine whether an instructive anterior patterning gradient has evolved in those beetle lineages in which there has been an increase in the number of segments patterned in the blastoderm prior to gastrulation (Patel *et al.* 1994).

(b) *Molecular transitions at the posterior egg pole: changes in the regulation of pair-rule gene homologues*

The primary pair-rule genes (*even-skipped*, *hairy* and *runt*) are the first genes within the *D. melanogaster* segmentation cascade to be expressed in a spatially periodic pattern of stripes (Jaynes & Fujioka 2004); see figure 2. The primary pair-rule genes activate a

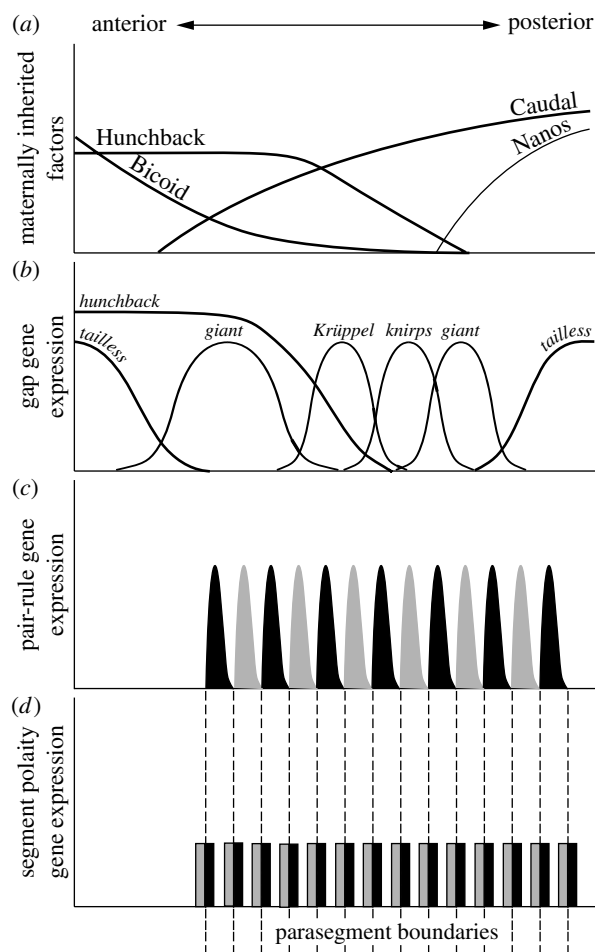


Figure 2. The *Drosophila melanogaster* segmentation gene cascade. (a) *Maternal genes*. Maternal transcripts of the segmentation genes *caudal* and *hunchback* are uniformly distributed, whereas maternal *bicoid* mRNA is tethered to the anterior pole of the egg. Localized at the posterior pole is a complex of maternal proteins and RNAs that includes transcripts of the gene *nanos*. On fertilization, maternal mRNAs are de-repressed, and Bicoid and Nanos proteins diffuse from their sources of production to form gradients within the egg. Bicoid activates zygotic *hunchback* expression and represses *caudal* translation in the anterior, whereas Nanos represses the translation of maternal *hunchback* in the posterior. As a result Hunchback protein is restricted to the anterior of the egg and protein gradients of Bicoid (decreasing posteriorly) and Caudal (decreasing anteriorly) form. In parallel, a maternally encoded terminal patterning system operates during embryogenesis; the product of *torso-like*—which is expressed within specialized follicle cells situated at both egg poles during oogenesis—catalyses the localized cleavage of a protein encoded by *trunk* within the perivitelline fluid. The trunk cleavage product acts as a ligand on the receptor tyrosine kinase encoded by *torso*, triggering a signalling cascade that regulates the zygotic expression of downstream segmentation genes, such as *tailless*, at either pole of the egg. (b) *Gap genes*. The net result of maternal signalling is the activation along the egg antero-posterior axis of a series of zygotic gap genes (i.e. *giant*, *Krüppel*, *tailless*), named so because their mutation leads to gaps in the region of the embryo in which they are normally expressed. The protein products of the gap genes themselves diffuse within the syncytial blastoderm, regulate each other and thus further refine their expression. Gap genes also play an important role at this stage in regulating the expression of the Hox genes, whose protein products confer identity to segments. (c) *Pair-rule genes*. In the next tier of the *Drosophila* segmentation cascade are three genes—*even-skipped*, *runt* and *hairy*—whose expression is driven by the maternal and gap gene transcription factor products. All three genes possess complex regulatory sequences that interpret the aperiodic expression of maternal and gap gene products and drive expression in a periodic pattern of seven stripes. These genes are collectively referred to as pair-rule genes since their mutation often leads to abnormalities in alternate segments. The three ‘primary’ pair-rule gene products in turn regulate expression of ‘secondary’ pair-rule genes, such as *fushi tarazu*, *paired*, *sloppy-paired* and *odd-skipped*. Black curve, *even-skipped*; grey curve, *fushi tarazu*. (d) *Segment polarity genes*. The pair-rule gene products activate the final tier in the *Drosophila* segmentation gene cascade, the segment polarity genes. These are the genes encoding proteins that actually initiate the formation of segment boundaries, and, as the name suggests, confer polarity to segments. Segment polarity genes are expressed in a series of 14 stripes, with odd and even stripes regulated by a different combination of the pair-rule proteins. The boundary between the expression of two of these genes, *engrailed* and *wingless*, becomes the parasegmental boundary, whereas segment boundaries form later, posterior to *engrailed* expression (adapted from Peel *et al.* 2005). Black bar, *engrailed*; grey bar, *wingless*.

suite of secondary pair-rule genes, which includes *paired*, *sloppy-paired* (1 and 2) and *odd-skipped* (Jaynes & Fujioka 2004). They are collectively referred to as pair-rule genes since their mutation often leads to abnormalities in alternate segments. Recent work on *T. castaneum* has revealed divergent

regulatory interactions between the homologues of *D. melanogaster* pair-rule genes. Choe *et al.* (2006), show that it is the homologues of *D. melanogaster even-skipped*, *D. melanogaster runt* and *D. melanogaster odd-skipped* that comprise the primary tier of pair-rule genes in *T. castaneum*. Surprisingly, rather than

Table 1. A summary of some *T. castaneum* and *N. vitripennis* segmentation gene homologues shown by recent studies to exhibit differences in expression and function when compared with *D. melanogaster* (see figure 2).

gene	<i>T. castaneum</i>	<i>N. vitripennis</i>
<i>bicoid</i> ; Stauber <i>et al.</i> (2002)	<ul style="list-style-type: none"> — <i>bicoid</i> not present in the genomes of insects outside the cychlorrhaphan flies — evolved from a <i>zerknüllt</i> (<i>zen</i>)-like precursor — Orthodenticle-1 appears to play an analogous role to <i>D. melanogaster</i> Bicoid in these species 	
<i>orthodenticle-1</i> ; Schröder (2003), Lynch <i>et al.</i> (2006a) and Olesnický & Desplan (2007)	<ul style="list-style-type: none"> — mRNA is maternally inherited—unlike in <i>D. melanogaster</i> where expression is purely zygotic—but not localized to the egg poles as in <i>N. vitripennis</i> — maternal plus zygotic expression becomes anteriorly restricted — functions with Hunchback to pattern the anterior of the embryo 	<ul style="list-style-type: none"> — unlike in <i>D. melanogaster</i>, maternal mRNA is localized to the anterior and posterior poles (via distinct mechanisms at either pole) — on fertilization, mRNA is released and diffuses to form opposing anterior and posterior mRNA and, through translation, protein gradients — anterior gradient functions with maternal Hunchback to activate anterior gap genes: <i>empty spiracles</i>, <i>giant</i> and (zygotic) <i>hunchback</i> — posterior gradient functions with Caudal to pattern posterior segments — largely conserved zygotic head gap gene role
<i>giant</i> ; Bucher & Klingler (2004), Brent <i>et al.</i> (2007) and Olesnický & Desplan (2007)	<ul style="list-style-type: none"> — no maternal expression as is the case in <i>D. melanogaster</i> — expressed in two zygotic gap-like domains as in <i>D. melanogaster</i>, except that the posterior domain is positioned much more to the anterior — anterior domain controls segment identity via the regulation of Hox genes, but unlike in <i>D. melanogaster</i>, it is not required for segment formation — required for the formation of all thoracic and abdominal segments, not just the segments in which it is expressed 	<ul style="list-style-type: none"> — unlike in <i>D. melanogaster</i>, maternal mRNA is localized to the anterior during oogenesis — on fertilization, mRNA is released and diffuses to form anterior mRNA and, through translation, protein gradient— represses central gap gene <i>Krüppel</i> in anterior, preventing repression of anterior gap gene <i>hunchback</i> by <i>Krüppel</i>, and thus plays a permissive role in anterior development — a similar zygotic gap gene role to <i>D. melanogaster</i>
<i>caudal</i> ; Schulz <i>et al.</i> (1998), Wolff <i>et al.</i> (1998), Copf <i>et al.</i> (2004), Lynch <i>et al.</i> (2006a), Olesnický <i>et al.</i> (2006), Olesnický & Desplan (2007)	<ul style="list-style-type: none"> — maternal mRNA initially uniformly distributed as in <i>D. melanogaster</i> — posterior protein gradient forms through translational repression by an unknown factor/ factors (i.e. not Bicoid) — expressed in the cellularized growth zone — unlike in <i>D. melanogaster</i>, required for the formation of all but the most anterior few segments 	<ul style="list-style-type: none"> — unlike in <i>D. melanogaster</i>, maternal mRNA is localized to posterior during oogenesis — on fertilization, is mRNA released and diffuses to form an mRNA and, through translation, protein gradient — functions with Orthodenticle-1 to pattern posterior via activation of posterior gap genes — influence extends further to the anterior than in <i>D. melanogaster</i> and includes activation of central gap gene <i>Krüppel</i>
<i>tailless</i> and the terminal patterning system; Schröder <i>et al.</i> (2000), Schoppmeier & Schröder (2005) and Lynch <i>et al.</i> (2006a,b)	<ul style="list-style-type: none"> — <i>tailless</i> expressed by a small group of cells at the posterior pole of the blastoderm — in contrast to <i>D. melanogaster</i>, there is no expression at the anterior pole of the blastoderm — terminal patterning system (<i>torso</i> and <i>torso-like</i>) required for sequential segmentation and formation of anterior extraembryonic tissue 	<ul style="list-style-type: none"> — <i>tailless</i> expression is activated in anterior and posterior by Orthodenticle-1 (i.e. there is no evidence for a terminal patterning system in <i>N. vitripennis</i>) — unlike <i>D. melanogaster</i>, the anterior domain is not required for segmentation — posterior domain has more extensive influence on posterior patterning than in <i>D. melanogaster</i>

canonical pair-rule phenotypes, the individual knock-down of each of these genes results in asegmental phenotypes in which all but a few anterior segments are deleted. The authors disrupted the expression of

each primary pair-rule gene in turn using parental RNA interference (RNAi) and then examined the expression of the remaining genes in knock-down embryos. Although direct regulatory interactions were

not proven, the results suggested to the authors that *T. castaneum even-skipped* activates *T. castaneum runt*, which in turn activates *T. castaneum odd-skipped*, which completes a regulatory cycle by repressing *T. castaneum even-skipped*. On the basis of these data, it was proposed that the genes comprise a regulatory gene circuit, each cycle of which sequentially patterns pairs of segments via the downstream regulation of a secondary tier of pair-rule gene homologues composed of *T. castaneum paired* and *T. castaneum sloppy-paired* (N.B. *T. castaneum hairy* does not appear to play a role in trunk segmentation; Choe & Brown 2007).

This model and the data it is based on raise an interesting question. How could a regulatory circuit of transcription factors—that by definition must operate intracellularly—pattern segments within a cellularized zone of extension? One possibility I suggest is that the proposed transcription factor circuit—or perhaps just some components of it—constitute an intracellular molecular oscillator with an analogous role to the molecular oscillators that control sequential segmentation of the presomitic mesoderm during vertebrate development (see reviews by Pourquié 2004; Gridley 2006). The vertebrate segmentation clock relies on the Notch intercellular signalling pathway, both as a component of the molecular oscillator in some cases (Pourquié 2004; Gridley 2006), and to coordinate oscillations among neighbouring cells (Masamizu *et al.* 2006). Work on myriapods and chelicerates has shown that some pair-rule gene homologues and members of the Notch intercellular signalling pathway are expressed in a dynamic fashion during sequential segmentation in a manner reminiscent of that seen during vertebrate sequential segmentation (Stollewerk *et al.* 2003; Chipman *et al.* 2004; Schoppmeier & Damen 2005), leading to the exciting hypothesis that a segmentation clock, analogous if not homologous to that operating in vertebrates, controls sequential segmentation in these arthropods (Peel & Akam 2003; Stollewerk *et al.* 2003). However, as yet, there is no evidence for the involvement of the Notch-signalling pathway during insect sequential segmentation. Wingless signalling also plays a central role in the vertebrate segmentation clock (Pourquié 2004; Gridley 2006;). Perhaps wingless signalling forms the basis to a possible segmentation clock in insects (Miyawaki *et al.* 2004) or alternatively other signalling pathways might be involved.

In *D. melanogaster* the periodic expression of primary pair-rule genes is, somewhat curiously, activated by an aperiodic series of antero-posteriorly restricted domains of gap gene expression (figure 2). Gap genes play an additional role in *D. melanogaster* development; they regulate the antero-posteriorly restricted domains of Hox gene expression which confer identity to segments (Irish *et al.* 1989). In *T. castaneum*, most *D. melanogaster* gap gene homologues are expressed in restricted antero-posterior domains, consistent with a gap gene function, and in a roughly conserved antero-posterior order, albeit shifted towards the anterior (Schröder 2003; Bucher & Klingler 2004; Cerny *et al.* 2005). However, the knockdown by RNAi of at least two of the *D. melanogaster* gap gene homologues—*Krüppel* and *giant*—does not result in canonical gap gene phenotypes, i.e. the loss of the segments in and around their domains

of expression (Bucher & Klingler 2004; Cerny *et al.* 2005). Instead, these segments take on abnormal identities as a result of the misexpression of Hox genes (Bucher & Klingler 2004; Cerny *et al.* 2005). This might imply that one of the ancestral roles of gap gene homologues in insects was to position Hox gene domains correctly, and that they were only later recruited to pattern pair-rule genes (Peel & Akam 2003). Under this model, gap gene recruitment is correlated with the transition to activating pair-rule stripes simultaneously in a syncytium, where control by intercellular signalling becomes redundant and where a spatial rather than temporal regulatory input is required (Peel & Akam 2003). Presumably transcription factors expressed at the right time and place in the posterior blastoderm were co-opted to regulate progressively more posterior pair-rule stripes, thus explaining the complex nature of the regulatory sequence of *D. melanogaster* primary pair-rule genes. And perhaps the co-option of *D. melanogaster* gap gene homologues was favoured, since they had already evolved a spatially and temporally corresponding role in Hox gene regulation.

4. MOLECULAR TRANSITIONS UNDERLYING THE CONVERGENT EVOLUTION OF LONG GERM EMBRYOGENESIS

The four major holometabolous insect orders all contain species exhibiting long germ embryogenesis, for example, the dipteran *D. melanogaster*, the lepidopteran *Manduca sexta*, the coleopteran *Callosobruchus maculatus* and the hymenopterans, *Apis mellifera*, *N. vitripennis* and *Bracon hebetor* (Grbic & Strand 1998; see also Davis & Patel 2002). However, the Lepidoptera, Coleoptera and Hymenoptera also contain species that have retained, or re-evolved in the case of parasitic hymenopterans (Grbic & Strand 1998), differing degrees of sequential segmentation, for example, the lepidopteran *Bombyx mori*, the coleopteran *T. castaneum* and the hymenopteran *A. ervi* (Grbic & Strand 1998; Davis & Patel 2002). This has led to the idea that long germ development evolved multiple times independently during the holometabolous insect radiation (see Davis & Patel 2002). This scenario now seems more likely due to a recent re-evaluation of holometabolous insect phylogeny. The general consensus surrounding the relationship of the major holometabolous insect orders used to be that the Diptera and Lepidoptera are sister groups, and that the Coleoptera form the basal branch in the tree (Whiting 2002). However, a recent molecular phylogenetic study supports a reversal in the position of the Hymenoptera and Coleoptera, such that the Hymenoptera now form the basal branch (Savard *et al.* 2006). Thus, the Diptera and Hymenoptera are now separated by the Coleoptera which contains many species with clear sequential segmentation.

Work by the Desplan and Pultz laboratories has begun to reveal the molecular basis to segmentation in *N. vitripennis*, which has an embryonic fate map almost identical to that of *D. melanogaster* (Bull 1982; Brent *et al.* 2007). If *N. vitripennis* and *D. melanogaster* did evolve long germ embryogenesis independently, as the latest molecular phylogenies suggest, comparisons

between these two species are a first step to determining the extent to which underlying gene network changes were also convergent.

As in *D. melanogaster* (figure 2), the segmentation cascade operating in *N. vitripennis* can be divided into four distinct tiers of maternal, gap, pair-rule and segment polarity genes. The major genetic differences identified so far between *D. melanogaster* and *N. vitripennis* are found right at the top of the segmentation cascade and relate to changes in the maternal contribution to patterning. These differences are summarized in table 1 and are discussed below.

In *D. melanogaster*, anterior development is largely under the control of the Bicoid morphogen gradient (Lawrence 1992); figure 2. However, *bicoid* is known to be an invention of the higher Diptera (Stauber *et al.* 2002). In *N. vitripennis* anterior patterning is accomplished by two distinct anterior gradients of patterning molecules (Lynch *et al.* 2006a; Brent *et al.* 2007). In contrast to *D. melanogaster*, where their expression is purely zygotic, in *N. vitripennis* maternal mRNAs of both *orthodenticle-1* and *giant* are tethered to the anterior pole during oogenesis (for details see Lynch *et al.* 2006a; Brent *et al.* 2007; Olesnick & Desplan 2007). Following fertilization these mRNAs are translated to form anterior gradients of Orthodenticle-1 and Giant protein. The mechanism by which maternal protein gradients form in *N. vitripennis* is slightly different from that of *D. melanogaster*—rather than proteins diffusing from localized sources of mRNA, on fertilization mRNAs are released from the egg pole and diffuse to form mRNA gradients that are converted into protein gradients via translation. The maternal Orthodenticle-1 gradient functions to activate anterior segmentation genes, such as the gap genes *empty spiracles*, (zygotic) *giant* and *hunchback* (Lynch *et al.* 2006a), while the maternal Giant gradient functions to set the anterior expression boundary of the central gap gene *Krüppel* (Brent *et al.* 2007). The repressive role of maternal Giant is permissive for anterior development, since, in its absence, *Krüppel* expression spreads anteriorly to repress the anterior gap gene *hunchback* (Brent *et al.* 2007).

The influence of Orthodenticle-1 and Giant on embryonic patterning does not extend as far to the posterior as does Bicoid in *D. melanogaster* (Lynch *et al.* 2006a; Brent *et al.* 2007). Instead, the influence of the posterior determinant *caudal* extends further towards the anterior—unlike in *D. melanogaster*, *N. vitripennis caudal* activates the central gap domain of *Krüppel* (Olesnick *et al.* 2006). Indeed, posterior patterning in *N. vitripennis* exhibits significant differences when compared with *D. melanogaster*. A posterior gradient of Caudal is established in *N. vitripennis*—in the absence of translational repression by Bicoid (figure 2)—via the tethering of *caudal* mRNA to the posterior pole during oogenesis (Olesnick *et al.* 2006; Olesnick & Desplan 2007). The mRNA of *orthodenticle-1* is also tethered to the posterior pole during oogenesis, such that, together, gradients of Caudal and Orthodenticle-1 protein control posterior patterning (Lynch *et al.* 2006a; Olesnick *et al.* 2006).

The large degree of variation between *D. melanogaster* and *N. vitripennis* in maternal patterning suggests that the gene network changes underlying the independent

evolution of long germ embryogenesis within the hymenopteran and dipteran lineages might have been very different. However, many of the observed differences could be attributed to the evolution of a maternal Bicoid gradient within the higher Diptera (Stauber *et al.* 2002). Indeed, *bicoid* and *orthodenticle* are both homeobox-containing genes, and Bicoid is predicted to have usurped the role of Orthodenticle as an anterior determinant (as exemplified by *T. castaneum* (Schroder 2003) and *N. vitripennis* (Lynch *et al.* 2006a) *orthodenticle-1*; table 1) through gaining affinity for Orthodenticle DNA binding sites via the convergent acquisition of a lysine residue at position 50 in its homeodomain (Treisman *et al.* 1989). Further data from additional long germ dipterans (e.g. *Apis gambiae*) and hymenopterans (e.g. *A. mellifera*) will be required to map divergent (and convergent) gene network changes to the dipteran and/or hymenopteran lineages. For example, is the maternal anterior patterning role of the homologue of the *D. melanogaster* gap gene *giant* a character that evolved specifically within the hymenopteran lineage, or a character that was lost during the dipteran radiation within the lineage leading to *D. melanogaster*?

There does, however, already appear to be a strong case for differences in the evolution of the terminal patterning system during the independent evolution of long germ development within the hymenopteran and dipteran lineages. In *D. melanogaster* a terminal patterning system acts to determine the most anterior and posterior regions of the embryo (Lawrence 1992); figure 2. The product of the gene *torso-like* is maternally restricted to the egg poles where it cleaves the protein encoded by the gene *trunk* (Casali & Casanova 2001). The Trunk C-terminal then acts as a ligand, binding to the receptor tyrosine kinase encoded by *torso* and triggering a signalling cascade that regulates the zygotic expression of downstream segmentation genes, such as *tailless* (table 1), at either pole of the egg (Casali & Casanova 2001). In *T. castaneum*, both *torso* and *torso-like* are required at the posterior pole for the activation and/or maintenance of sequential segmentation (Schoppmeier & Schroder 2005). However, in *N. vitripennis* the expression of *tailless* has been shown to be dependent on the anterior and posterior gradients of maternal Orthodenticle-1 and thus perhaps not a terminal patterning system (Lynch *et al.* 2006b). The absence of a *D. melanogaster*-like terminal patterning system in hymenopterans is further supported by the failure to find homologues of *torso* or *trunk* in the *A. mellifera* genome (Dearden *et al.* 2006).

The apparent lack of a *Drosophila*-like terminal patterning system in long-germ hymenopteran insects is intriguing, particularly since one appears to be operating during sequential segmentation in *T. castaneum* (Schoppmeier & Schroder 2005; Lynch *et al.* 2006b). Studies on more holometabolous insects, as well as non-holometabolous insects, will be required to determine if a terminal patterning system is a character that has been lost within the hymenopteran lineage, or gained in the lineage leading to the other major holometabolous insect orders. One possibility is that in some of the insect lineages that underwent the transition to long germ embryogenesis (i.e. in dipteran lineages) a terminal patterning system that ancestrally played an important

role in initiating or maintaining sequential segmentation was co-opted to activate posterior segmentation genes (Schoppmeier & Schroder 2005), whereas in other lineages undergoing parallel transitions (i.e. in hymenopteran lineages) this did not occur.

5. GENERAL CONCLUSIONS

(a) *The role of co-option in the evolution of segmentation gene networks*

A theme emerging from comparative studies on insects is the role that co-option plays in evolution, at different levels of complexity. At the regulatory sequence level, it seems possible, if not likely that some gap gene homologues—expressed at the right time and in the right place due to an ancestral role in Hox gene regulation—have been co-opted to regulating pair-rule gene homologues, perhaps via the simple acquisition of binding sites (Bucher & Klingler 2004; Cerny *et al.* 2005; Choe *et al.* 2006). At the protein level, Bicoid—or perhaps more accurately Zerknullt—was co-opted to an anterior patterning role within the higher Diptera via a simple coding mutation that allowed it to recognize the regulatory targets of an existing anterior determinant; Orthodenticle (Stauber *et al.* 2002; Lynch *et al.* 2006a). At the intracellular level, existing cytoskeletal machinery may have been co-opted during the evolution of instructive anterior patterning gradients (Bucher *et al.* 2005). All these cases are consistent with a long series of simple modifications to developmental gene circuits having, over evolutionary time, underpinned diversification at the morphological level. The results of a recent whole genome study are consistent with the widespread occurrence of gene co-option during insect evolution. Dearden *et al.* (2006) looked at the presence/absence of homologues of *D. melanogaster* developmental genes in the *A. mellifera* genome. They found that of the developmental genes involved in processes that are known to be divergent between these two species (i.e. sex determination, dosage compensation, meiosis and germ-cell development), a significant number of those conserved in *A. mellifera* ($\chi^2 = 19.03$, $p < 0.001$, $n = 78$) have multiple (i.e. pleiotropic) functions in *D. melanogaster*. This suggests either that the homologues of genes with multiple functions in *D. melanogaster* have been lost less frequently in the honeybee lineage and/or that genes with ancestral conserved functions have been frequently co-opted into new roles in the fruitfly lineage (Dearden *et al.* 2006).

(b) *The evolution of developmental gene networks in relation to adult morphology*

The comparative studies reviewed in this paper clearly demonstrate that genetic networks controlling the development of conserved adult structures (i.e. homologous insect segmental units) can diverge significantly over time due to lineage specific transitions in cellular embryology associated with changes in life-history strategy. They also suggest that distinct gene network changes might underlie convergent transitions in modes of cellular embryogenesis. This implies that over the course of hundreds of millennia, developmental gene networks and the adult morphology they pattern can become ‘decoupled’. Assigning homology, or otherwise,

to adult morphological features based on comparative developmental genetic data alone is therefore risky. Accurately reconstructing the evolution of animal body plans will require an holistic approach, which includes adequate and intelligent sampling of species (i.e. perhaps less focus on species exhibiting highly derived modes of embryogenesis), a more thorough understanding of the embryological contexts in which gene networks operate and a better appreciation of how evolutionary changes in life-history strategy (i.e. changes in species ecology) can influence the evolution of development.

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