Arthropod Limbs and their Development

11

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Contents

11.1 Introduction

Arthropods are characterized by bodies that are segmented and by the possession of paired ventral limbs carried on all, most or some of these body segments. These paired limbs are primitively segmented—and the name of the taxon Arthropoda refers to the jointed limbs of its members. While the origin of arthropods is not the focus of this chapter, it is relevant to note that the recent discovery of a Cambrian lobopodian, *Diania cactiformis*, possessing robust and probably sclerotized appendages with what Liu et al. (2011) interpret as articulating elements, led them to speculate whether arthropodization (sclerotization of limbs) preceded arthrodization (sclerotization of the body). In such a scenario, the acquisition of jointed limbs assumes centre stage as the key driver of arthropod evolution.

Historically, the intellectually intriguing task of reconstructing the evolutionary history of the arthropods has revolved around advances in understanding of structural diversity along two morphological axes: the tagmatization or functional division of the body along the antero-posterior (A-P) axis, and the segmentation and specialization of the jointed limbs along their proximo-distal (P-D) axis. Evolutionary trends along these two axes were separated by Boxshall (2004) in order to facilitate a morphological comparison of limbs between taxa exhibiting different tagmosis. However, limb specialization

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reflects a developmental process that commences with specification of segmental identity along the A-P axis and is intimately bound up with the major developmental pathways that regulate tagmatization (Averof and Patel 1997; Mahfooz et al. 2007).

The spectacular diversity of limb morphology has long been regarded as a key component of the amazing adaptive radiation of the Arthropoda and our knowledge of the developmental patterning mechanisms that generate this diversity is expanding rapidly (see Pechmann et al. 2010; Angelini et al. 2011). The task of integrating data from developmental genetics and morphology is guided by our understanding of phylogenetic relationships and the iterative process of estimating phylogenies has been reinvigorated by the flood of molecular data from next generation sequencing. The availability of sequence data on a massive scale is not only transforming the phylogenomics of arthropods (Regier et al. 2010), but has also facilitated the application of some of the powerful new tools of developmental genetics. In particular, "knock-down" methods using RNA interference (RNAi) have allowed us to test the roles of specific genes more directly. No longer is it necessary to set up cultures and endlessly screen progeny for mutants of particular genes: now, we can directly interfere with the expression of a specific gene and observe the consequences (e.g. Liubicich et al. 2009; Mito et al. 2011). In addition, the discovery of important new fossil arthropods has continued (Siveter et al. 2007a, b; Zhang et al. 2007; Briggs et al. 2012) and the application of novel techniques for extracting fragmentary microfossils (Harvey and Butterfield 2008; Harvey et al. 2012) has widened our understanding of the morphological diversity of early Palaeozoic arthropods.

The primary goal of this chapter is to integrate the wealth of new data emerging from morphological and embryological studies, from novel fossils, and from developmental genetics, in order to address questions of interest to the communities of scientists involved in the study of arthropod morphology and phylogenetics. Answers to these questions will help us to begin to formulate a new understanding of the spectacular diversity in limb diversity structure that has been the key to their success.

11.2 The Distinction Between Segments and Annuli

Arthropod limbs are subdivided along the P-D axis into smaller units, either segments or annuli. The anatomical distinction between segments and annuli in arthropod limbs was emphasized by Boxshall (2004): true segments are characterized by the presence of intrinsic muscles that originate, insert or attach within the segment whereas annuli lack intrinsic muscle origins, intermediate attachments or insertions. Intrinsic muscles or their tendonous extensions may, however, pass through annuli to an insertion site in a more distally located segment (Fig. 11.1). Each articulation is typically

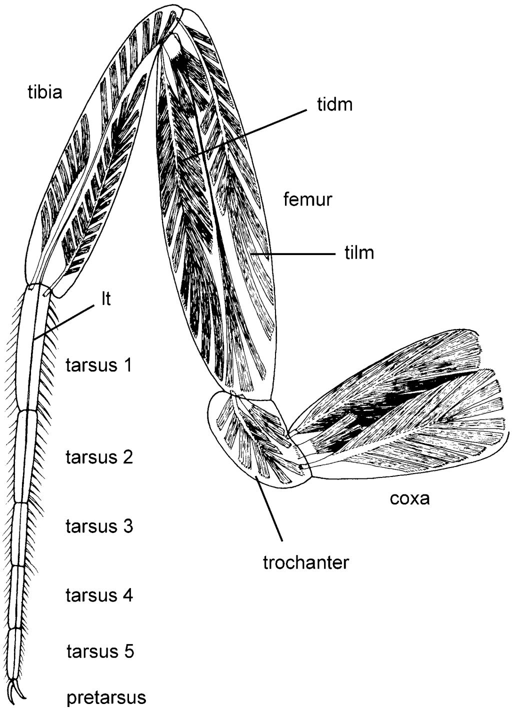

Fig. 11.1 Schematic representation of adult *Drosophila* leg, showing intrinsic muscles and tendons, including tibia levator muscle (*tilm*), tibia depressor muscle (*tidm*) and the long tendon (*lt*) passing through the tarsal annuli (based on data from Soler et al. 2004)

provided with a hoop of arthrodial membrane which allows telescoping of the proximal rim of the more distal segment within the distal part of the more proximal segment. The appropriate terminology for the subdivisions of the main P-D axis of an arthropod limb is dependent upon their anatomy: subdivisions may be referred to variously as segments or articles, annuli or annulations, and the neutral term podomeres is often used when anatomical information about musculature is lacking, as in the case of the majority of fossils. Both segments and annuli can sometimes be incompletely expressed, particularly during larval development.

Maruzzo et al. (2009) examined segmental mismatch in the naupliar antennal exopodite of the branchiopod crustacean *Artemia*. The exopodite carries a series of natatory setae along its posterior-ventral side with each, apart from the apical seta, located on a transverse cuticular fold. Along the anterior side of the ramus is a P-D series of incomplete ringlets or sclerites separated by joint-like cuticular folds. The two series are not in register and there were, on average, more ringlets than setae. This phenomenon was also noted in the naupliar exopodites of the antenna and mandible of representatives of a few other taxa, including some fossil branchiopods, some phosphatocopines, and an extant thecostracan. However, Maruzzo et al. (2009) showed that three exopodal muscles extend the length of the ramus and make intermediate attachments on both sides—in the ringlets (the anterior muscle) and in the setal-bearing cuticular folds (the two posterior muscles). Using the presence of intrinsic musculature as a rigid criterion, these naupliar rami could be regarded as multi-segmented, although the segments are incompletely expressed due to a decoupling of development in the two sides of the ramus analogous to the dorso-ventral decoupling in the development of diplopod body segments (see Damen et al. 2009).

Expressed segmentation can change significantly during development. In dendrobranchiate decapods, for example, the antenna of the naupliar and protozoeal phases initially has a multi-segmented exopodite (Fig. 11.2a). The exopodite gradually loses external segmentation until it has transformed into the characteristic, unsegmented antennal scale at the megalopa stage (Fig. 11.2b). The transition from segmented naupliar ramus to unsegmented antennal scale is unique to the caridoid malacostracans and is accompanied by a change in form of the endopodite, from a two-segmented ramus (Fig. 11.2a) to an annulate flagellum (Fig. 11.2c).

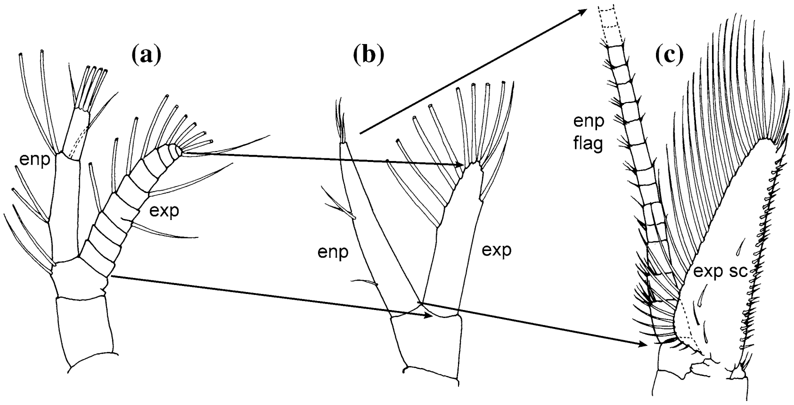

Fig. 11.2 The antenna of *Pleoticus muelleri* (Decapoda). **a** Protozoea III stage. **b** Mysis I stage. **c** Megalopa stage. Showing transition of exopodite (*exp*) from multi-segmented ramus to unsegmented antennal scale (*exp sc*), and of endopodite (*enp*) from two-segmented ramus to endopodal flagellum (*enp flag*) (redrawn from de Calazans 1992)

11.2.1 How are Segments Formed?

The early establishment of the P-D axis by the leg gap genes is a general feature of limb patterning during development in all arthropods (Angelini and Kaufman 2005; Williams 2008; Pechmann et al. 2010). However, the *Drosophila* leg is a useful comparative model since it comprises true segments proximally and tarsal annuli distally (Fig. 11.1). Leg formation in *Drosophila* depends upon the subdivision of the P-D axis into broad domains by leg gap genes: the early limb bud is subdivided into a distal domain expressing *Distal-less* (*Dll*) and a proximal domain expressing *extradenticle* (*exd*) and its co-factor *homothorax* (*hth*) (see Kojima 2004, for review). This proximal domain maintains expression of *hth* and *exd* and corresponds to the coxa and trochanter of the leg. Further differentiation along the P-D axis is mediated by the morphogens *Decapentaplegic* (*Dpp*) and *Wingless* (*wg*) which cooperate to induce the expression of *dachshund* (*dac*) in the intermediate region of the limb, between proximal and distal domains (Lecuit and Cohen 1997; Abu-Shaar and Mann 1998). These leg gap genes *hth*, *dac* and *Dll* control the formation of the proximal, middle and distal domains along the P-D axis, respectively (Fig. 11.3).

Downstream of the leg gap genes, the Notch signalling pathway plays a central role in segmentation along the P-D axis of the leg (de Celis et al. 1998; Bishop et al. 1999). The process of formation of true segments along the P-D axis of the limb of *Drosophila* takes place within the three leg gap gene domains, and the genes *Serrate* (*Ser*), *Delta* (*Dl*) and *fringe* are essential for joint formation (Rauskolb 2001; Mito et al. 2011). *Fringe* modulates Notch-ligand interactions (Panin et al. 1997). These induce expression of a set of transcriptional regulators that mediate joint morphogenesis and leg segment growth: *lines* and *bowl* act as a binary switch to generate a stable Notch signalling interface between *Dl*-expressing cells and adjacent distal cells (Greenberg and Hatini 2009).

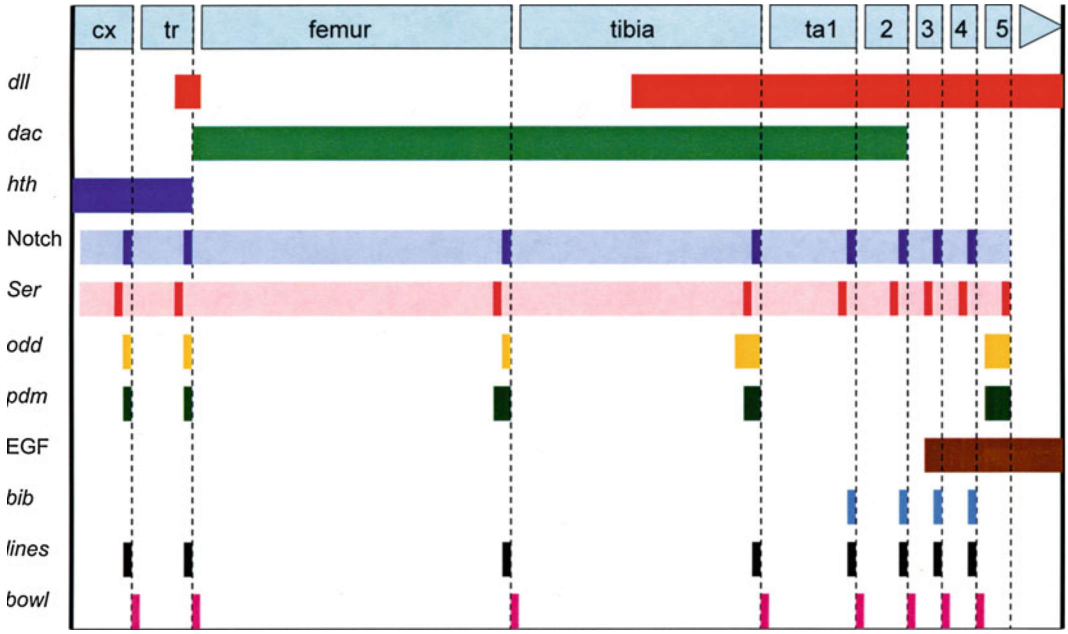

Fig. 11.3 Schematic showing expression domains of genes along P-D axis of *Drosophila* leg, compiled from various sources. Proximal end on *left* commencing with coxa (*cx*), trochanter (*tr*), femur and tibia, and with first to fifth tarsal annuli (*ta1–5*) on *right*. Names of genes given on vertical axis, and coloured shading shows expression domains

A number of other genes are also known to be involved downstream of Notch signalling, including *nubbin* (*pdm*) (Rauskolb and Irvine 1999), *odd*-related genes (Hao et al. 2003), *h/ Enhancer of split*-related genes (Bishop et al. 1999) and *Activator Protein*-2 (Kerber et al. 2001). Although all originally discovered in *Drosophila*, a mandibulate, orthologues of these genes have also been found in chelicerates and Prpic and Damen (2009) concluded that, despite minor differences, the mechanisms regulating leg segmentation are likely to have been conserved from the common ancestor of the Arthropoda.

11.2.2 How are Annuli Formed?

Intercalary annulation of the endopodite is widespread in arthropods, most commonly in the tarsal region (Boxshall 2004). In the chelicerates, extreme tarsal annulation is found in the antenniform first walking legs of amblypygids, which may comprise as many as 28 tibial and 54 tarsal annuli (Weygoldt 1996), and tri-annulate femurs and bi-annulate trochanters are also known. Some pycnogonids also have distally annulate pedipalps and first walking legs. Stenopodoidean and caridean crustaceans such as processed shrimps can have a multi-annulate carpus on the fourth pereopod which has a normal chela at its tip. The trunk limbs of scutigeromorph centipedes exhibit extensive annulation of the tarsal region, interpreted by Manton (1977) as an adaptation for rapid running. Most insects exhibit some annulation in the tarsal region, with the number of tarsal annuli varying from one to five as in *Drosophila* (Fig. 11.1). Bitsch (2001) considered the pentameric tarsus a possible apomorphy for the dicondylian hexapods, with secondary reductions responsible for the variation, as found in the Zygentoma, for example. In arthropod locomotory limbs, annulations are typically intercalary, although there are examples of terminal annulation, such as the flagellate swimming exopodites of the Mysidacea or Anaspidacea (Fig. 11.8c). Most examples of terminal annulation in arthropods involve sensory appendages, such as antennules and antennae (Fig. 11.2c).

In the *Drosophila* leg, there are five tarsal annuli and the patterning mechanism resulting in subdivision of the tarsus differs from that governing basic segmentation (Fig. 11.3). In the distal half of the leg is a zone of decreasing *dac* expression and increasing *Dll* expression extending from middle to tip of the leg. The genes *dpp* and *wg* together establish a secondary organizing centre towards the distal tip. Ligands from this centre activate the epidermal growth factor receptor pathway which controls the expression of the genes responsible for tarsal subdivision (Campbell 2002; Galindo et al. 2002). These tarsal genes, *bric-a-brac*, *apterous* and *BarH*1, act in combination with *dac* and *Dll*, to fine-pattern tarsal subdivision (see Greenberg and Hatini 2009). According to Greenberg and Hatini (2009), *lines* modulates the opposing expression landscapes of *dac* and the tarsal genes. Sharp boundaries in Dpp signalling trigger an episode of apoptosis that takes place during morphogenesis of tarsal joints in *Drosophila* (Manjón et al. 2007). Tarsal genes appear to be specific to the insects but little comparative research has been undertaken to either confirm their presence or determine the role of any orthologues in other arthropod taxa.

11.2.3 Is There a Difference in Timing of Appearance of Segments and Annuli During Development?

The distinction between limb segments and annuli is based on musculature. In the segmented antennules of copepods and ostracods, development follows a distal-to-proximal pattern with the articulations separating more distal segments typically appearing earlier than those separating the more proximal ones (Boxshall and Huys 1998; Smith and Tsukagoshi 2005). The adult antennules of copepods can possess up to 27 segments, and these are derived by a sequence of subdivisions of the three original segments present in the nauplius (Boxshall and

Huys 1998). The metamorphic moult from the sixth naupliar stage to the first copepodid stage was marked by the subdivision of the apical segment of the nauplius to form the distal eight segments of the adult antennule. No further subdivisions occur in this distal section throughout the subsequent moults. During the copepodid phase, the two proximal antennulary segments of the nauplius undergo a sequence of subdivisions to form segments 1 to 20 of the adult. Antennules with fewer expressed segments are envisaged as being generated by early cessation of the process of subdivision (Boxshall and Huys 1998; Schutze et al. 2000).

In limbs that possess a mix of segments and annuli, the segments tend to appear before the annuli. Unfortunately, *Drosophila* is not a good model here since both segments and annuli are everted simultaneously from the imaginal disc. In more basal hexapods such as symphypleone collembolans, Nayrolles (1991) showed that four true segments are initially expressed on the antennule; subsequently the distal segment undergoes annulation to generate the terminal flagellum (see Boxshall 2004: Fig. 1f–g). Minelli et al. (2000) showed that eosegments appear before merosegments in chilopod development and Boxshall (2004) considered this as analogous to the appearance of segments before annuli in other arthropods. In the decapod malacostracans, *Panulirus* and *Cherax,* the primary antennulary flagellum develops by the production of new annuli in a meristematic zone at the base of the flagellum (Sandeman and Sandeman 1996; Steullet et al. 2000). Subdivision takes place in annuli distal to the basal meristematic annulus and the process seems generally similar to that described for the endopodal flagellum of the antenna (i.e. the second antenna) of the isopod *Asellus*, which consists of a single segment divided into annuli devoid of intrinsic musculature (Wege 1911). The antennal flagellum comprises a proximal meristematic region, a central region composed of quartets (sets of 4 annuli each having a specific arrangement of setae), and an apical complex consisting of the apical annulus plus the four preceding annuli with specific setal patterns. The number of quartets in the central region is variable in *Asellus* since this isopod never ceases moulting and adds annuli throughout life (Maruzzo et al. 2007). The proximal meristematic annulus divides into a copy of itself (the meristem) and a distal annulus which is effectively an incomplete quartet,and divides following a set pattern each time, to produce the complete quartet. Maruzzo and Minelli (2011) found proximal growth zones on each of the elongate rami of the pleopods in amphipods. In these zones, new arthrodial membrane, separating newly differentiated annuli, and new setae were added during post-embryonic moults.

Proximal annulation is expressed transiently during the naupliar phase of some copepods but is lost by the first copepodid stage (Dahms 1992). Protozoeal larvae of some penaeid decapods similarly exhibit transient annulations in the proximal part of the antennule (Boxshall 2004: Fig. 2a-c), which are lost by the end of the zoeal phase. The proximal annulated part of the antennule of the fossil crustaceans *Rehbachiella* and *Bredocaris* may be interpreted as additional evidence of their larval status, but may also indicate that a proximal annulated zone is plesiomorphic for the Crustacea.

11.2.4 Are Segments Fundamentally Different from Annuli?

The patterning mechanisms generating segments and annuli are similar: knock-down of *Notch* in the cricket *Gryllus* resulted in a marked reduction in leg length and loss of joints along the P-D axis (Mito et al. 2011). The loss of joints is referred to as “fusion” by Mito et al. (2011) but is derived by failure of the joint to form and create a subdivision, rather than by fusion of subdivisions. Mito et al. (2011) found that the femur and tibia failed to separate and the tarsal annuli failed to subdivide normally, so Notch clearly plays a role in the formation of both segments and annuli. In contrast, *Dl* mutants of *Drosophila* showed shortened legs but only tarsal segments 2–4 of the wild type were not separated (Bishop et al. 1999). Similarly, knock-down of certain other genes is known to affect

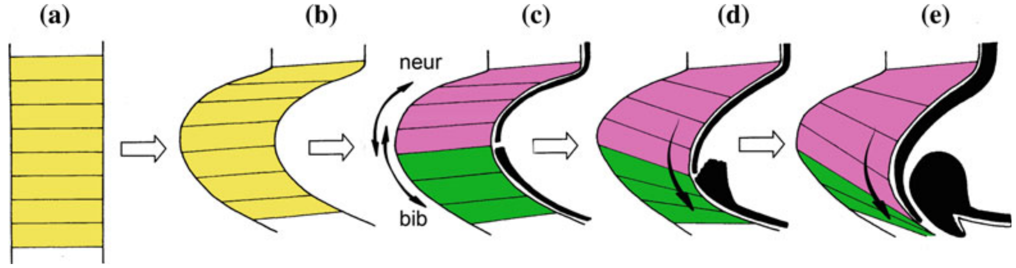

Fig. 11.4 Schematic showing morphogenesis of ball-and-socket tarsal joint in *Drosophila* leg. **a** Undifferentiated epithelial cells (*yellow*) of leg prior to formation of joint. **b** Initiation of joint formation by invagination of epithelium. **c** Epithelial cells differentiate into those with low Notch activity (*pink*) that will produce the socket and those with high Notch activity (*green*) that will produce the ball. **d** Migration of socket-producing epithelial cells begins. **e** Cell migration continues and lip-like projection develops on ball (adapted from Tajiri et al. 2011)

tarsal subdivision but not basic leg segmentation, so the patterning mechanisms for leg segments and leg annuli, while similar, exhibit important differences in detail (Fig. 11.3).

During normal development differentiation of a typical "ball-and-socket" joint between tarsal annuli in *Drosophila* is dependent upon levels of Notch activity. High Notch signalling levels promote ball production whereas low levels are required for socket production (Fig. 11.4). Cells that produce the ball express *big brain* (*bib*) whereas socket cells express *neur* and tend to produce the more uniform, thinner cuticle of the socket. Elongation of the ball lip and the socket coincides with the migration of the cells that form them. Notch activity is also required for this cell motility, but it is probably under the control of an independent Notch-mediated pathway (Tajiri et al. 2011). Interestingly, disruption of the Notch signalling pathway during pupal development in *Drosophila* suppressed production of the normal ball-and-socket joint in the legs and resulted in the formation of a more uniform type of joint like that found in more basal hexapods such as Ephemeroptera according to Tajiri et al. (2011).

Morphologically, the key difference between segments and annuli is the presence of intrinsic musculature in segments. A huge body of literature is available describing limb musculature patterns in a wide range of arthropods (see Manton 1977 and references therein), and the precise sites of muscle origins and insertions have been considered as phylogenetically informative (e.g. Boxshall 1997). However, the key challenge is to integrate knowledge of the anatomy with what is known about the genetic mechanisms regulating myogenesis in arthropods. Unfortunately, most studies on myogenesis in *Drosophila* have focused on larval and flight muscles, so relatively little is known about the mechanisms governing adult leg myogenesis in the *Drosophila* leg model. Soler et al. (2004) summarized the stages of myogenesis: commencing in the leg imaginal discs of the third instar: myoblasts expressing *twist* (*twi*) and located in the vicinity of tendon precursors start to express the muscle founder cell marker *dumbfounded* (*duf*). Subsequently, epithelial tendon precursors invaginate within the developing leg segments, giving rise to the tendons. Tendon associated *duf*-expressing muscle founder cells become distributed along these developing tendons and fuse with surrounding myoblasts forming syncytial myotubes. Finally, these myotubes grow towards their epithelial insertion sites (the apodemes) and complete the link between internally located tendons and the leg epithelium. However, the process is understood only in outline.

Leg muscle patterning involves genes such as *ladybird early*, which is expressed in a subset of the *twi*-expressing myoblasts located dorsally and ventrally in the femur and giving rise to the tibia levator and depressor muscles (Fig. 11.1), respectively (Maqbool et al. 2006). Only fragmentary data on mechanisms responsible for P-D patterning of leg musculature are available for other arthropods. Recent work on muscle precursors in the developing limbs of isopod and decapod crustaceans showed that intrinsic limb muscles originate from single precursor cells which subsequently form multi-nucleate precursors, and this suggests fundamental similarities with the insects (Kreissl et al. 2008; Harzsch and Kreissl 2010).

These outlines of the sequence of events involved in myogenesis shed little light on how significant spatial aspects (i.e. the precise location of muscle origins and insertions) of myogenesis are determined. However, Park et al. (1998) showed that muscle founder cells arise from progenitor cells which are singled out by a lateral inhibition process mediated by the Notch–Delta signalling pathway. Given the central role of the Notch pathway in segmentation along the P-D axis of the arthropod leg, it seems probable that spatial regulation of muscle attachments is also linked to the existing framework of domains along the P-D axis of the limb.

11.3 Arthropod Limb Types

In a review, Boxshall (2004) concluded that there are two basic limb types in crown-group arthropods: a single-axis first cephalic appendage (the antennules/chelicerae) and biramous post-antennulary limbs. In the terminology of Scholtz and Edgecombe (2005), the first cephalic appendages of euarthropods represent the "secondary antennae", with innervation derived from the deutocerebrum, as distinct from the "primary antennae" associated with the protocerebrum and found in onychophorans (see Chap. 10). Scholtz and Edgecombe (2006) discuss possible fates for the missing "primary antennae" in euarthropods, but these are not of concern here. I am considering the first cephalic limb of arthropods, which is derived from the deutocerebral segment and is known as the antennule or first antenna in crustaceans, the antenna in insects, myriapods and trilobites, chelicera in crown-group chelicerates and the "great appendage" in megacheirans.

11.3.1 The First Cephalic Limb

Interpretations of antennules as possessing vestiges of an "exopodite" still crop up occasionally in crustacean taxa such as the podocope ostracods (Karanovic 2005; Marmonier et al. 2005) but lack credible supporting evidence according to Boxshall et al. (2010) who also concluded that the Remipedia, with an antennule comprising a single primary axis composed of segments (defined by the possession of intrinsic musculature), plus a proximally located ventral flagellum, and the Malacostraca, with a short segmented primary axis bearing two, occasionally three, distally located flagella, provide no evidence that contradicts the inference that the antennules of the Mandibulata are primitively single-axis limbs. This single axis may be either segmented, flagellate or a mix of segments and annuli but is essentially modular in construction, and this modularity confers important functional attributes, permitting, for example, the enhancement of a sensory array by the addition of modules or by the specialization of individual modules independent of others.

The first prosomal appendages of crown-group chelicerates are the paired chelicerae. The comparison of expression patterns of Hox genes in chelicerates and mandibulates has demonstrated that chelicerae are positional homologues of the antennules (Damen et al. 1998; Telford and Thomas 1998; Abzhanov et al. 1999), and the immunohistochemical analysis of neuroanatomy and neurogenesis has confirmed the deutocerebral derivation of the chelifores of pycnogonids (Brenneis et al. 2008).

The morphological gulf between an elongate sensory antennule and a short feeding chelicera seems profound, but recent analyses of cheliceromorph fossils have hypothesized how such transitions might have occurred (Fig. 11.5b–e). These analyses involve the Megacheira, the so-called short great appendage fossils, which are possible stem-group chelicerates (e.g. Chen et al. 2004; Cotton and Braddy 2004). The antennules of the megacheiran *Leanchoilia* (Fig. 11.5b) were considered as effectively triflagellate by

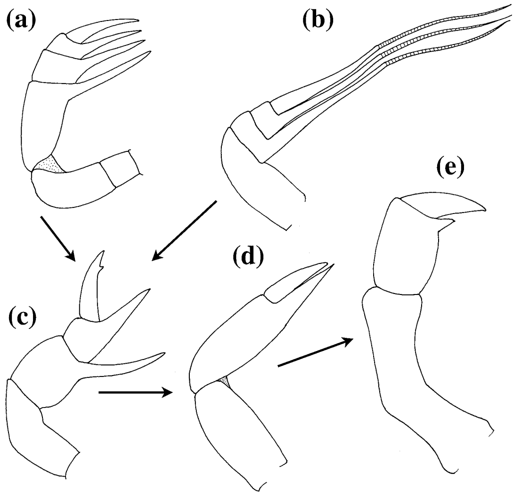

Fig. 11.5 Schematic showing possible transition between raptorial great appendage and chelicera. **a** Great appendage of *Yohoia*. **b** Great appendage of *Leanchoilia*. **c** Great appendage of *Haikoucaris*. **d** Chelicera of *Limulus*. **e** Chelicera of pycnogonid (**a**, **c**, redrawn from Haug et al. (2012), **b**, redrawn from Edgecombe et al. (2011), **d**,**e**, drawn from photographs in Haug et al. (2012)

Boxshall (2004), but each flagellum is borne on a rigid spinous projection of the antennulomere (Bruton and Whittington 1983). In the evolutionary scenario constructed by Haug et al. (2012), the megacheirans, *Parapeytoia*, *Fortiforceps*, *Yohoia* (Fig. 11.5a) *Leanchoilia* and *Haikoucaris* (Fig. 11.5c), are all considered to be derivatives of the stem lineage of the Chelicerata, and a transition from triflagellate great appendage to chelicera is hypothesized as involving reduction and loss of the flagella, reduction and loss of segments, shortening of the spinous projections and the development of a special “elbow joint” between the two-segmented peduncle and the distal segments (Fig. 11.5a–e). However, this scenario needs further testing firstly because it was not supported by the phylogenetic analysis of Edgecombe et al. (2011), which recovered a monophyletic Megacheira as the sister-group of a poorly resolved group comprising chelicerates, aglaspids and other fossil cheliceromorph taxa such as *Cheloniellon* and *Sidneyia*. Secondly, the Silurian synziphosurine *Dibasterium durgae* has long flexible antenniform chelicerae (Briggs et al. 2012) providing an elegant link between typical sensory antennule and feeding chelicerae.

Although bi-, tri- or multi-flagellate limbs are known in malacostracan crustaceans and in basal megacheirans, a truly biramous first limb (with two-segmented axes) is unknown in the Arthropoda. The only possible exception might be the Pauropoda which have two-branched antennules, but each branch is unsegmented and provided with musculature that inserts only around its proximal rim (Boxshall 2004: Fig. 2g) and so does not comprise a segmented axis.

The first cephalic limb of euarthropods has a single P-D axis and thus differs from post-antennulary limbs which are primitively biramous. How fundamental is this distinction, given that well-known homeotic mutations, such as the Antennapedia mutant of *Drosophila*, indicate that antennules and post-antennulary limbs can be viewed as serial homologues? Indeed, numerous homeotic mutations are now known that can transform maxillary palps, labial palps and genitalia into antennae or thoracic legs in a variety of insects, not just *Drosophila* (Angelini et al. 2011).

Less is known about patterning mechanisms in the developing arthropod antennule than in legs, but it is clear that early development is regulated by the activity of field-specific selector genes. The *Drosophila* antenna comprises only three segments and a terminal flagellate section, the arista. On the basis of gene expression domains, Postlethwait and Schneiderman (1971) concluded that the first antennal segment was “homologous” with the coxa of the leg; the second segment with the trochanter; and the third with the femur, tibia and first tarsal segment, and the arista with the second to fifth tarsal segments plus the tarsal claw. However, the homology is at the level of the shared early leg gap gene patterning mechanism, common to all arthropod limbs, and does not support an inference of homology between the segments themselves.

The basic patterning mechanism of the antenna is very similar to that of the leg, but differs in the extensive co-expression of the proximal and distal leg gap genes, *hth* and *Dll,* respectively, and in the absence of a functional intermediate domain specified by *dac* (Dong et al. 2001) (the *dac* expression domain lies completely within the *Dll* domain in the insect antenna). Downstream of the leg gap genes, the Notch pathway involving *Dl* has been reported for the antenna of the cricket *Gryllus* (Mito et al. 2011). Fine-scale mechanisms are also somewhat similar: the gene *lines*, for example, plays analogous roles in the subdivision of the flagellate arista of the antenna and of the annulate tarsal region on the leg, but again there are also some significant differences (Greenberg and Hatini 2009). In particular, in the antenna, *Dll* and *hth* cooperate in a secondary role, to impose identity on the antenna by activating antenna-specific genes in a cascade leading to *distal antenna*, a selector gene for antennal fate (Emerald et al. 2003).

There is no evidence from gene expression data to suggest that antennules (or chelicerae) are primitively anything other than single-axis limbs. So, for example, in the early embryo of the extant xiphosuran *Limulus,* the developing limb buds of the chelicerae do not develop a second lateral point of *Dll* expression even though transient laterally located expression points are shown by the developing buds of all the post-antennulary limbs on the prosoma, including pedipalps and walking legs (Mittmann and Scholtz 2001). There is a difference of interpretation concerning the homology of these transient *Dll*-expressing points (see Boxshall 2004) but the evidence relevant here is that the chelicerae lack such a point.

The shared common features between antenna and leg development in *Drosophila* indicate that, despite some significant differences, the antennules and post-antennulary limbs of all arthropods can be viewed as serial homologues, but specification of the anterior-most limb as the antennule ensures that it develops as a single axis rather than biramous limb.

11.3.2 The Post-antennulary Limbs

Boxshall (2004) concluded that the basic post-antennulary limb of crown-group arthropods comprises an undivided protopodite (also called the basipod), an endopodite of cylindrical segments and a more flattened exopodite probably of two segments.

11.3.2.1 Protopodite

The protopodite is the proximal part of the biramous limb and carries the rami. It is easy to recognize in biramous limbs, as found in crustaceans, trilobites and many other fossils such as the marrellomorph *Xylokorys*, but when limbs are uniramous, it can be difficult to identify the boundary between the protopodite and the endopodite (see Boxshall 2004).

The protopodite of all post-antennulary limbs of trilobites and most fossil and recent chelicreomorphans is entire and undivided, although a small, mobile proximal endite is present in xiphosurans, eurypterids and the Cambrian *Sidneyia* (a relative of *Aglaspis* according to the scheme in Edgecombe et al. 2011). In trilobites, the entire medial margin of the undivided protopodite was convex and provided with spines, forming a gnathobase. Similar undivided gnathobases are also retained on the pedipalps and walking limbs of *Limulus*, the pedipalps of spiders, in the first and second walking legs of scorpions and some harvestmen. The retention of protopodal endites (often referred to as gnathendites) in these taxa was a plesiomorphic character state in the analysis of Shultz (1990). The protopodite of chelicerates and cheliceromorphs in general appears to be short but very broad. However, the discovery of the cheliceromorph *Dibasterium* has revealed a biramous prosomal limb type in which the endopodite is carried on a recognizable protopodite but the well-developed and multi-segmented exopodite appears to originate separately on the adjacent ventral surface of the prosome (Briggs et al. 2012). The limbs of *Offacolus* were reinterpreted as similar to those of *Dibasterium* by Briggs et al. (2012).

In crustaceans, as representatives of basal mandibulates, the protopodites are more elongate in the P-D axis and retain gnathobases or endites in many members of the limb series: for

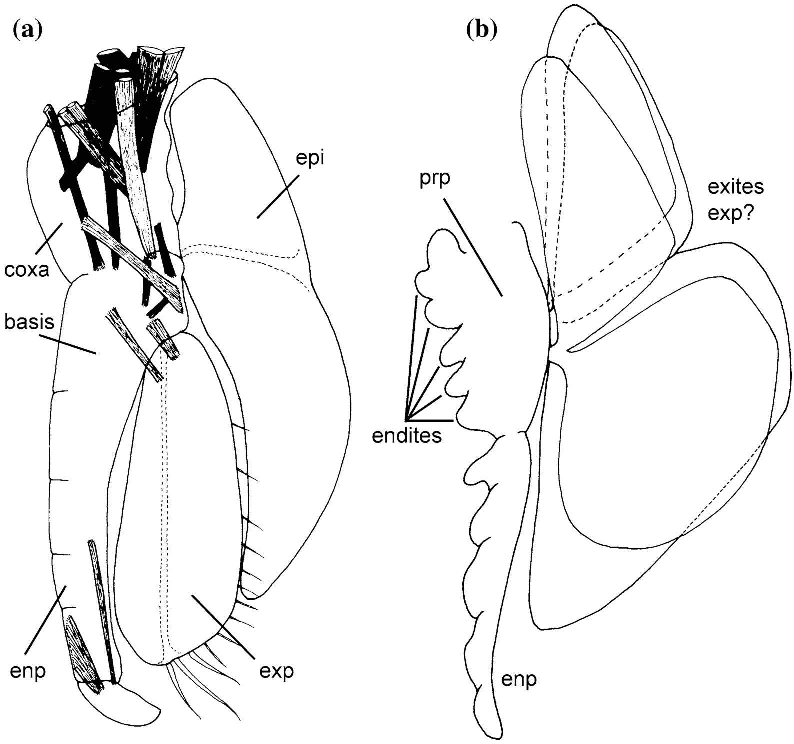

Fig. 11.6 Trunk limbs of leptostracan and archaeostracan Malacostraca. **a** Trunk limb of *Nebalia* showing absence of endites in protopodal part (coxa and basis), musculature in exopodite (*exp*) and endopodite (*enp*) but none in the foliaceous epipodite (*epi*). **b** Trunk limb of *Cinerocaris* showing enditic margin of protopodite (*prp*) and array of foliaceous outer lobes (**a**, from Boxshall and Jaume (2009); **b**, redrawn from Briggs et al. (2004))

example, in mandibles, in the post-mandibular limbs of crustaceans such as cephalocaridans and branchiopods and in the maxillule of hexapods, chilopods and symphylans. Endites may also be transient features: the enditic process on the coxa of the antenna of planktotrophic crustacean nauplii is secondarily lost after the naupliar phase of development. In crustaceans, the medial surface of the enlarged protopodite typically carries a linear series of endites. The number of endites on the protopodite of post-mandibular limbs varies: in Cambrian crustaceans such as *Rehbachiella* and *Dala,* it can be up to eight or nine (Walossek 1993; Walossek and Müller 1998). Only five or six endites are retained on the protopodal part of the trunk limbs of *Lepidocaris* and five endites has often been regarded as typical for extant branchiopods. However, Pabst and Scholtz (2009) regarded only three of the inner lobes as protopodal in origin, reinterpreting the two distal lobes as endopodal. The enditic margin only forms weak lobes in cephalocaridans rather than well-defined endites. The archaeostracan *Cinerocaris* retains a series of endites on the undivided protopodite of the pereopods (Fig. 11.6b) and has endite-like expansions of the medial margin of the proximal endopodal segments (Briggs et al. 2004). Retention of an endite series along the protopodite was regarded by Walossek (1999) as characteristic of his Entomostraca, but is plesiomorphic for malacostracans also. The endites are lost in modern leptostracans (Fig. 11.6a).

Outside the crown-group crustaceans, only one endite per segment is typical. The lacinia and galea of the maxilla (first post-mandibular limb) in the basal hexapod *Thermobia* have been interpreted as representing the endites of two protopodal segments (Chaudonneret 1950). However, in *Tribolium,* two enditic lobes are present transiently in the early embryo but fuse before hatching to form the single endite present in the larva. This larval endite is presumed to give rise to the lacinia and galea of the adult (Jockusch et al. 2004), however, fusion and subsequent separation of endites derived from different protopodal segments seems unlikely.

Chaudonneret (1950) studied *Thermobia* but no relevant detailed genetic studies have yet been carried out on this species. Most hexapods and myriapods lack functional endites on their limbs.

The proximal endite on the protopodite of crustaceans has been regarded as of particular significance by Waloszek and co-authors (e.g. Maas et al. 2003; Waloszek 2003; Waloszek et al. 2007). As summarized by Waloszek et al. (2007, p. 284), the "proximal endite" is a "novelty of the ground pattern of the Crustacea" and is a "separately moveable" setose lobe "nested within the ample joint membrane medially below the basipod of the post-antennular limb". The proximal endite is clearly visible on the post-antennulary limb series in *Martinssonia* (Müller and Walossek 1986) and in the phosphatocopines (Maas et al. 2003) but is presented only in the mandible in *Oelandocaris* (Stein et al. 2005). The significance of this proximal endite in the phylogenetic debate is that it "is considered as a phylogenetic precursor of another limb portion developed in the Crustacea"—the coxa (Waloszek et al. 2007).

An alternative hypothesis, as summarized by Boxshall (2004), is that the protopodite (Waloszek's basipod) subdivided by the formation of a transverse articulation to form the proximal coxa and distal basis. This must have occurred in the stem lineage of the mandibulates at least in the antenna (first post-antennulary limb) and mandible (second), as well as in the maxillule (third) of crown-group Crustacea according to Boxshall (1997). In the maxilla and post-cephalic trunk limbs, the proximal endite is simply the proximal-most of the series of endites expressed along the medial margin of the protopodite. It may be capable of performing motions independent of the main promotor-remotor swing of the whole limb at the body-coxa articulation, but such multi-functionality is the hallmark of the crustacean limb.

Do gene expression data shed any light on the debate over the origin of the separate coxa and basis? Endites can express *Dll*. In the developing uniramous limbs of chelicerates and insects, a proximal zone of expression of *Dll* is found. It is localized in the gnathendite on the undivided protopodite (the coxa) of the developing pedipalps of the mygalomorph spider *Acanthoscurria*, although not in the rudimentary gnathendites of the walking legs (Pechmann and Prpic 2009). Similar expression in the gnathendite of the pedipalps has also been observed in more derived spiders (Schoppmeier and Damen 2001; Prpic and Damen 2004). In insects, *Dll* is expressed on the maxilla of *Tribolium* in a distinct proximal domain that corresponds with the developing endite (Beermann et al. 2001) and in *Acheta*, in two domains corresponding with lacinia and galea (Angelini and Kaufman 2004). Interestingly, RNAi depletion of *Dll* did not affect the formation of the endites (the galea and lacinia) on the maxilla of another beetle, *Onthophagus*, although the palp became unsegmented (Simonnet and Moczek 2011).

In the phyllopodial limbs of anostracans, Williams (2008) demonstrated early *Dll* expression in the proximal regions of the limb in the series of endites carried on the medial margin, around the margins of both endopodite and exopodite, and in the pre-epipodite. Transient expression only was noted for the epipodite (which lacks setae in the adult anostracan). Williams (2008) noted that proximal *Dll* expression was found initially in general epithelial cells but subsequently became localized to setal-forming cells, irrespective of whether the setae were sensory or had a passive mechanical role as in the majority of enditic setae.

In the notostracan *Triops,* there is medially reiterated expression of *dac* in the very early limb bud that resolves to the endites. Each of the five endites carried along the medial margin of the *Triops* trunk limb (Fig. 11.7c) expresses *dac* in a zone along its lower (ventral) margin (Sewell et al. 2008). Localized *dac* expression was also noted in each endite on the trunk limbs of an anostracan branchiopod (Sewell et al. 2008). Four zones of *dac* expression were observed along the margin of the maxilla of the myriapod *Glomeris* (Prpic and Tautz 2003) and *dac* was also expressed in both endites present on the maxilla of the hexapod *Tribolium* (Prpic et al. 2001). Interestingly, the gnathendite of the

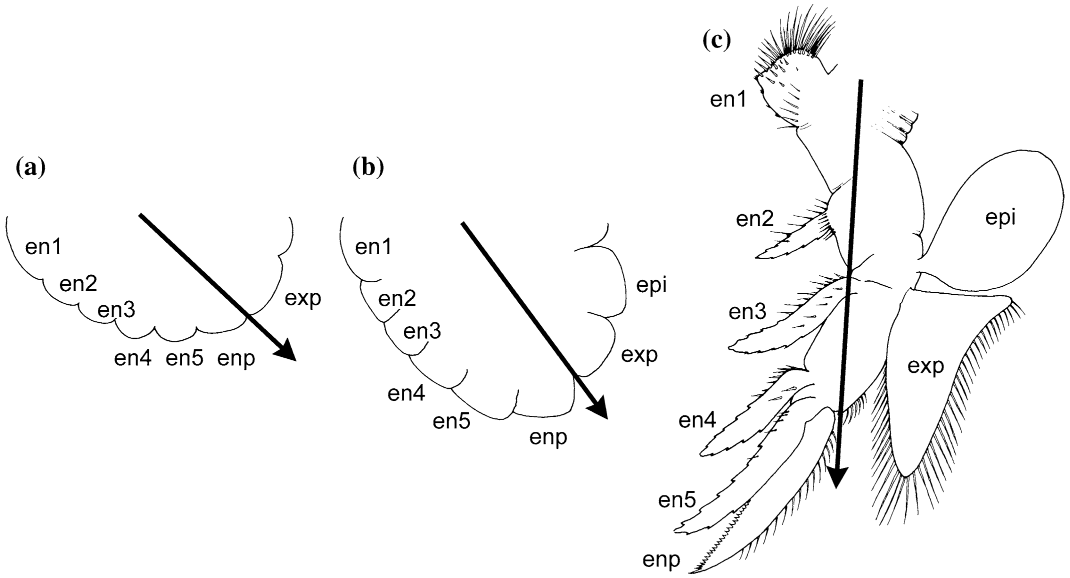

Fig. 11.7 Development of trunk limb of Notostraca. **a** Schematic showing limb forming as transverse ridge with developing endites and rami as defined as lobes. **b** Later stage of limb development with epipodite lobe now present. **c** Adult limb of *Lepidurus* showing endites, rami and epipodite. Endite 4 (*en 4*) and endite 5 (*en 5*) plus endopodite (*enp*) of Sewell et al. (2008) were all interpreted as representing a tripartite endopodite in the scheme of Pabst and Scholtz (2009) (**a**, **b**, adapted from Sewell et al. (2008); **c**, redrawn from Sars (1896))

pedipalps of the model chelicerate *Cupiennius* lacks *dac* expression. Sewell et al. (2008) considered this as evidence consistent with the inference that the proximal endites of mandibulates and cheliceromorphs are non-homologous (Boxshall 2004).

These expression data can help us understand the derivation of the coxa and basis from an undivided ancestral protopodite in two respects. Firstly, the P-D subdivision of limbs is regulated by a patterning mechanism involving the leg gap genes, the Notch signalling pathway, and a downstream cascade of other genes, which is common to all arthropods. All subdivisions of the P-D axis appear to be regulated by this mechanism, and there is no evidence to suggest that the coxa-basis articulation in the mandibulate protopodite is different. I infer that the coxa and basis differentiate by a process of subdivision, as for every other segment and annulus along the limb, and that there is no special role for the proximal endite. Secondly, the proximal endite is one of a P-D series of protopodal endites, all of which share a common expression pattern for the few genes (e.g. *dac* and *exd*) that have thus far been investigated. No unique expression pattern has yet been noted for the proximal endite: again there is nothing to suggest that the patterning mechanism responsible for the formation of this endite is different from that of the more distal endites in the series.

The enlarged proximal endite of the second post-antennulary limb in the Mandibulata is modified as a gnathobase. Indeed, possession of the second post-antennulary limb modified as a mandible has been used to characterize the Mandibulata, comprising the Crustacea, Hexapoda and Myriapoda (Snodgrass 1938). The limb carried on the homologous body segment in chelicerates is a walking leg (Damen et al. 1998; Telford and Thomas 1998)—the first walking leg in arachnids and the second in Xiphosura and Eurypterida. In trilobites and other fossils with homonomous post-antennulary limbs, this limb exhibits no unique morphological specializations—resembling all other members of the series.

The mandibles of hexapods, myriapods and adult malacostracan and branchiopod crustaceans are gnathobasic and protopodal in origin (Popadić et al. 1996; Prpic et al. 2001), lacking a palp. The protopodal origin of the mandible in hexapods is confirmed by lack of *Dll* expression, and in myriapods by transient *Dll* expression (Popadić et al. 1996). The gnathobasic origin of the mandible in branchiopods, cephalocaridans, remipedes and malacostracans is not in question because they all possess a distal palp earlier in development. Most other crustacean taxa either retain a mandibular palp as an adult or lose the palp after the naupliar phase. Loss of the mandibular palp is shared with the Hexapoda and Myriapoda.

The mandibular gnathobase in Crustacea is formed from the proximal segment only (i.e. the coxa) of the two protopodal segments. The ostracod mandible with a basal endite as well as the coxal gnathobase is an exception (Boxshall 2004: Fig. 9b). When present, the palp comprises the distal protopodal segment (the basis) plus the rami. The mandible is homologous to all members of the Mandibulata, so the mandibular gnathobase is formed by the coxa only in hexapods and myriapods as well. In chelicerates and trilobites, the second post-antennulary limb has an undivided protopodite. The coxal gnathobase of the mandibulate mandible is not homologous with the gnathobase of the second post-antennulary limb of cheliceromorphs which is derived from the medial margin of the entire protopodite (as pointed out by many authors, see Boxshall 2004).

11.3.2.2 Endopodite (= Telopodite)

There has been considerable confusion and debate concerning the number of endopodal segments in the phenotypic ground plan of each major arthropodan taxon, and numerous schemes have been proposed to establish homologous landmarks along the P-D axis of the various limbs. Manton (1966) referred to the “welter of assumptions” underpinning such schemes, and the key problem is that within every major arthropodan class, there is marked variation in number of endopodal segments expressed in the phenotype, so uncertainty remains despite the considerable attention devoted to this topic.

Numbers of apparent segments can be larger than a hypothesized ground plan due to subdivision of segments. In the diplopods, for example, the trunk legs were described by Manton (1954, 1958) as having a seven-segmented endopodite consisting of trochanter, pre-femur, femur, post-femur, tibia, tarsus and claw (pretarsus); however, the coxa and trochanter of Manton represent two annuli of a subdivided segment, and the femur and post-femur of Manton similarly represent a subdivided segment. Similarly, in some mysid malacostracans, for example, pereopodal endopodites have been described as having a total of six segments, with a pre-ischium located between the basis and ischium (e.g. Hansen 1925). This is also a secondary increase.

Oligomerization—the reduction in number of expressed limb segments in the phenotype—also seems to have been a common evolutionary trend in limb segmentation within taxa. The loss of segments typically results from failure of expression of articulations during development rather than from actual fusion (Boxshall and Huys 1998). These are different processes although both result in a compound segment originating from two or more ancestral segments. Articulations between true segments may fail to be expressed and in such cases, the plane of the ancestral articulation may be marked externally by a suture line in the integument, and/or internally by a muscle insertion or by the retention of a transverse tendonous section within a muscle (Boxshall 1985), or may be lost entirely.

The endopodite of branchiopod trunk limbs has often been interpreted as secondarily unsegmented, but new data on the development of *Limnadopsis* led Pabst and Scholtz (2009) to suggest that the endopodite is fundamentally three-segmented. They consider there to be good evidence supporting the view that a tri-partitite endopodite (either three-lobed or three-segmented) is the general pattern for the

Branchiopoda, as proposed earlier by Hansen (1925). By analogy with such an interpretation, the trunk limb of *Triops* would also have a tripartite endopodite (Fig. 11.7c).

It would be convenient if the wealth of emerging data on gene expression patterns were to provide any marker that could be used to unequivocally identify specific joints along the P-D axis to serve as landmarks for comparison between taxa. However, this seems unlikely since the comparative data that are available show homologous patterning domains do not necessarily mark homologous morphological domains (Abzhanov and Kaufman 2000; Sewell et al. 2008). There are, however, markers for very specific cellular functions which may be localized in particular limb parts, such as the epipodites.

In the biramous post-cephalic trunk limbs of barnacles (Crustacea: Thecostraca), the rami are transformed into cirri that form the food capture apparatus of the sessile adult. These cirri extend hydraulically and but flex using their intrinsic musculature (Cannon 1947). The intrinsic muscles form an intermediate attachment in each segment of the cirrus, indicating that these subdivisions are true segments rather than annuli. Similarly, both rami of the antenna of conchostracan crustaceans comprise multiple podomeres and appear flagellate, but both comprise segments defined by the presence of intrinsic muscles (Boxshall 2004: Fig. 8g). Such examples of secondary increases in true segmentation are relatively rare. Where both rami of a limb are similar and secondarily multi-segmented, as in the antennae of conchostracan branchiopods and the thoracopodal cirri of barnacles, the P-D patterning mechanism is presumably the same for both rami. In the pleopods of amphipod crustaceans, for example, both rami continue to add articulations in a proximal growth zone, at each post-embryonic moult (Maruzzo and Minelli 2011). In such cases, it can be inferred that the secondary segmentation would be controlled by a single, specialized patterning mechanism common to both rami.

11.3.2.3 Exopodite

Exopodites on post-antennulary limbs are a feature of the arthropod ground plan (Walossek 1999). The exopodite is the outer ramus and has a distal origin on the protopodite, lateral to the endopodite. It is typically provided with muscles originating in the protopodite and inserting within the ramus itself and when the exopodite is often two-segmented, the intrinsic musculature can move the segments relative to one another. Boxshall and Jaume (2009) looked at the diversity of exopodites, noting the prevalence of subdivided exopodites in branchiopods, branchiurans and cephalocaridans, but considered that the basic euarthropodan exopodite was two-segmented. However, multi-segmented exopodites are found in crustacean naupliar limbs (antennae and mandibles), in trunk limbs of copepods, thecostracans and remipedes, in certain phosphatocopines, and in *Agnostus*.

Foliaceous exopodites are present on the trunk limbs of branchiopods (Fig. 11.8b) and of most Palaeozoic fossil arthropods for which the limbs are known, including mandibulates such as *Bredocaris*, *Cinerocaris*, *Dala*, *Rehbachiella* and *Tanazios*; trilobites such as *Burgessia*, *Eoredlichia*, *Misszhouia* and *Olenoides*; and fossils of uncertain affinity such *Sapeiron* (see references in Boxshall 2004 and Boxshall and Jaume 2009). Indeed, arthropods with a series of uniramous post-antennulary limbs lacking exopodites, such as the Silurian pycnogonid *Haliestes* (Siveter et al. 2004), are the exception in the early to mid-Palaeozoic. The rare case of the fossil arthropod *Sarotrocercus* which apparently retains the exopodite only (see Boxshall 2004) may be better interpreted as lacking information on the endopodite (Haug et al. 2011). Within the extant Crustacea, each post-antennulary limb from the antenna to the uropod is biramous somewhere in crustacean morphospace. The exopodite is often lost from particular adult limbs, although larvae may retain an exopodite even if the adults secondarily lack one. In the Eumalacostraca, the distal segment of the pereopodal exopodite has been regarded as

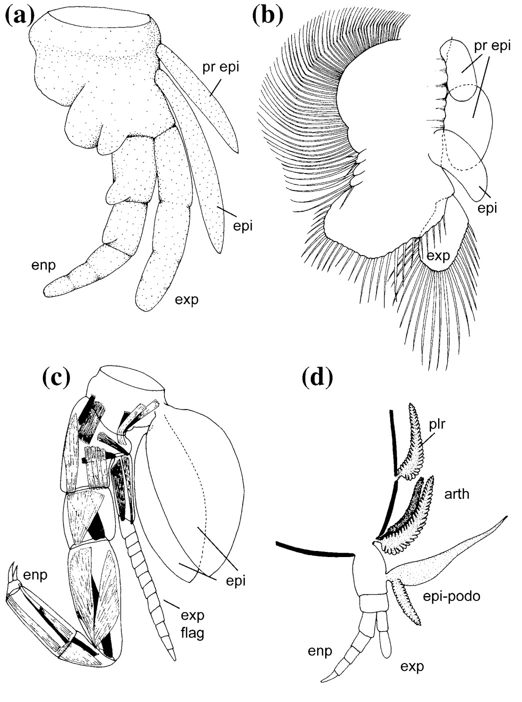

Fig. 11.8 Diversity of biramous trunk limbs bearing epipodites. **a** *Tanazios* showing blade-like epipodite (*epi*) and pre-epipodite (*pr epi*) and cylindrical endopodite (*enp*) and exopodite (*exp*). **b** *Polyartemia* showing epipodite (*epi*), two pre-epipodites (*pr epi*) and foliaceous exopodite. **c** *Anaspides* showing double epipodite (*epi*) plus flagellate exopodite (*exp flag*). **d** schematic showing arthrobranch (*arth*), and pleurobranch (*plr*) gills, and epipodite-podobranch complex of dendrobranchiate decapod (**a**, simplified drawing from reconstruction in Siveter et al. (2007b), with enditic membranes omitted; **b**, redrawn from Sars (1896); **c**, adapted from Boxshall (2004); **d**, adapted from Boxshall and Jaume (2009))

primitively annulated (Fig. 11.8c) (Boxshall and Jaume 2009); however, the presence of muscles extending the length of the flagellate exopodite in larval decapods (Harzsch and Kreissl 2010) highlights the importance of obtaining better data for basal taxa such as the syncarids, the development of which is poorly documented. The exopodite is not expressed in extant hexapods and myriapods, so inferences on the form of the exopodite in the Mandibulata necessarily depend on evidence from the Crustacea and related fossils. The Silurian *Tanazios* has been interpreted as a probable stem-lineage crustacean (Siveter et al. 2007b) and as a labrophoran (Boxshall 2007), and it has slender type of segmented exopodite on its trunk limbs (Fig. 11.8a).

The Silurian marrellomorph *Xylokorys* is of particular interest: it has a well-developed, single-axis antennule, followed by the first to fourth post-antennulary limbs each of which has a well-developed multi-segmented exopodite. The first and second post-antennulary limbs have exopodites comprising a basal part of two or three podomeres and a distal section of four or five podomeres carrying a conspicuous setal fan (Siveter et al. 2007a). In the third post-antennulary limb, the endopodite is reduced and the exopodite is very large with a distal section of up to 7 podomeres, each bearing a setal tuft (Fig. 11.9a).

This distinctive type of exopodite closely resembles that found in the Silurian cheliceromorph *Offacolus*, the second to fifth post-antennulary limbs of which each have a six-segmented exopodite (Fig. 11.9b) terminating in a setal fan (Sutton et al. 2002). *Dibasterium* also has a robust multi-segmented exopodite on the same prosomal limbs and Briggs et al. (2012) concluded that the exopodite of both *Offacolus* and *Dibasterium* inserts on the body surface separate from the endopodite-bearing protopodite.

The enigmatic Cambrian arthropod *Ercaia* has a very similar first post-antennulary limb, with an exopodite comprising a segmented cylindrical proximal part plus a flattened distal part bearing a conspicuous setal array (Chen et al. 2001). The presence of a well-developed, articulated cylindrical exopodite in these taxa suggests that this may represent a second basic exopodite type in Palaeozoic arthropods, in addition to the foliaceous type of exopodite.

Foliaceous exopodites are retained on the more posterior trunk limbs in these taxa and in other cheliceromorphs such as *Sanctacaris* and *Limulus* (Boxshall 2004: Fig. 4c), and other marrellomorphs such as *Marrella* and *Mimetaster*. Interestingly, *Xylokorys*, *Offacolus* and *Dibasterium* have the endopodites of the first few pairs of post-antennulary limbs terminating in a subchela. There appears to be a similar structure of the anteriormost pairs of limbs between these

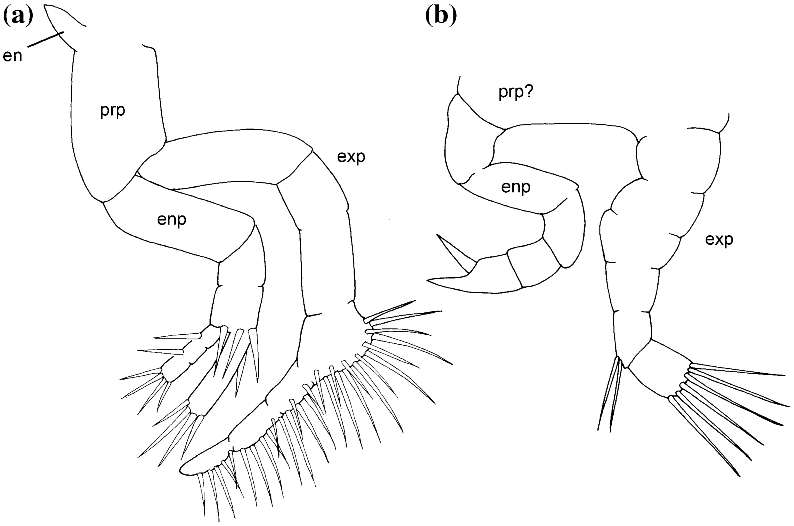

Fig. 11.9 Anterior post-antennulary limbs from Silurian arthropods with well-developed exopodites. **a** Third post-antennulary limb of the marrellomorphan *Xylokorys*, showing well-developed cylindrical exopodite (*exp*) with distal part bearing setal array, and segmented endopodite with subchelate apex. **b** Post-antennulary limb of cheliceromorphan *Offacolous*, showing two multi-segmented rami, with setal tuft on apex of exopodite (*exp*) (**a**, drawn from reconstructions in Siveter et al. (2007a); **b**, redrawn from Sutton et al. (2002)). The form of the protopodite is uncertain (see Briggs et al. (2012))

two taxa, and, interestingly, both exhibit the biphasic arrangement of post-antennulary limbs into anterior and posterior homonomous blocks (Boxshall 2004).

Dll expression can be used to distinguish exopodites from lateral outgrowths, such as epipodites, which result from the establishment of new lateral axes. These usually do not express *Dll*. For example, the exopodal nature of the scaphognathite on the decapod maxilla (in the freshwater crayfish, Astacida) was confirmed by Scholtz et al. (2008) on the basis of such evidence. The earliest expression of *Dll* is in the tip of a crustacean limb bud irrespective of the form of the adult limb, that is, whether it is biramous or uniramous, stenopodial or phyllopodial (Olesen et al. 2001; Williams 2004; Wolff and Scholtz 2008). The endopodite and exopodite are formed by a secondary subdivision of the primary growth zone at the tip of the developing P-D limb axis (Wolff and Scholtz 2008). The subdivision of the primary limb axis is reflected by the transformation of the initially undivided *Dll* expression at the tip of the limb bud into two separate *Dll* domains representing the tips of the rami (Williams 2004; Wolff and Scholtz 2008). The mechanism producing this subdivision is unknown but likely scenarios are the suppression of *Dll* expression in the area between exopodal and endopodal domains, or apoptosis (Wolff and Scholtz 2008).

The loss of the exopodite from the thoracopods of the haplopodan branchiopod *Leptodora* resulted from suppression of the bifurcation of the early limb bud (Olesen et al. 2001). Wolff and Scholtz (2008) showed that uniramous pereopods of the amphipod *Orchestia* are formed by the suppression of the split into exopodite and endopodite of the primary growth zone of the main limb axis. Comparing the clonal composition of the embryonic pereopods and pleopods, Wolff and Scholtz (2008) demonstrated that a population of cells with the identical genealogical background to that which forms the exopodite in the biramous pleopods contributes to the outer part of the endopodite of the uniramous pereopods along most of the P-D axis but not to the tip. Boxshall and Jaume (2009) interpreted the failure of expression of the exopodite in development as resulting in the cells that would have comprised the exopodite anlage being conscripted to contribute to the endopodite.

11.3.2.4 Epipodites and Pre-epipodites

The crustacean epipodite is a lateral outgrowth from the coxal part of the limb protopodite. Epipodites are found on the post-maxillary trunk limbs in branchiopods (Figs. 11.7c, 11.8b) and on the thoracopods (maxillipeds and pereopods) in the Malacostraca (Figs. 11.6a, 11.8c,d). Epipodites are characterized by the lack of musculature (Boxshall and Jaume 2009). Transient rudiments of epipodites were also reported during the development of the anterior pleopods of the Leptostraca by Pabst and Scholtz (2009). Epipodites are rarely found on cephalic limbs within the extant crustaceans: exceptions include the presence of a setose lobate epipodite on the maxillule of copepods (Huys and Boxshall 1991) and the well-developed setose epipodite on the maxilla of the myodocopan ostracods. Myodocopans are the only crustaceans that possess an epipodite on the maxilla (Boxshall and Jaume 2009: Fig. 16).

In addition to the epipodite, a more proximally located pre-epipodite is also present in most anostracan Branchiopoda (Fig. 11.8b) and within the Malacostraca—in Leptostraca (Fig. 11.6a) and the Silurian archaeostracan *Cinerocaris* (Fig. 11.6b). Two pre-epipodites are present in chirocephalid Anostraca (Fig. 11.8b), and in other anostracans, the pre-epipodite shows clear evidence of a double origin (Williams 2007). Adult *Anaspides* has two very similar epipodites originating immediately adjacent to each other on the pereopodal coxa (Fig. 11.8c). Although neither shows any evidence of a double origin, one could represent the pre-epipodite. However, the presence of a single coxal epipodite in the Carboniferous *Palaeocaris* and in the bathynellaceans suggests the possibility that the presence of two lobes in *Anaspides* is a secondarily derived state within the Syncarida (Boxshall and Jaume 2009).

The epipodite typically appears very early in development as an unarmed, rounded lobate bud, and in the Branchiopoda (Fig. 11.7a–c), where post-maxillary limbs initially appear as transverse ridges, the epipodite bud appears just prior to the limbs commencing their swing down to the vertical, adult orientation (Møller et al. 2004). This pattern is common to anostracan and notostracan branchiopods. In leptostracan malacostracans, the epipodite on the pereopods appears somewhat later in development of the limbs, as the swing to vertical is taking place (Pabst and Scholtz 2009).

Ungerer and Wolff (2005) showed that the coxal plate and epipodite of amphipod pereopods arise from a common anlage in early development and considered it possible that the coxal plate of amphipods might be homologous with the pre-epipodite. Boxshall and Jaume (2009) questioned the widely assumed homology of the peracaridan oostegite with the pre-epipodite. Oostegites and pre-epipodites have different sites of origin on the protopodite, differ structurally, functionally and in orientation. More importantly, Boxshall and Jaume (2009) highlighted that oostegites are secondary sexual structures, often undergoing cyclical change in concert with the hormonally controlled, reproductive cycle of the female and hypothesized that their underlying genetic control mechanisms would also differ. Oostegites may well be a novel structure, apomorphic to the Peracarida.

The epipodite is characterized by distinctive gene expression patterns: strongly expressing *nubbin* (*pdm*), *apterous* (*ap*) (Averof and Cohen 1997), *trachealess* (Mitchell and Crews 2002) and *ventral veinless* (Franch-Marro et al. 2006), but only weakly expressing *Dll* in a transient manner (Williams 1998; Williams et al. 2002). Richter (2002) regarded the specific expression pattern of *pdm* and *ap* in the distal epipodite of *Artemia* and in the epipodite of *Pacifastacus* as a strong argument for homology of these two structures. Irrespective of shared ancestry, the expression of numerous genes by the epipodites of malacostracans and branchiopods probably reflects common functionality as osmoregulatory-gaseous exchange organ. Currently, there is little evidence available to suggest whether two pre-epipodites of chirocephalids or the double pre-epipodite of other anostracans are homologous with the pre-epipodite of anaspidacean malacostracans.

Boxshall (2004) concluded that epipodites on limb protopodites appeared relatively late in the Palaeozoic and were not present in the crustacean ground plan. The discovery of new fossils has challenged this conclusion: Zhang et al. (2007) reported "epipodites" on the trunk limbs of the Cambrian *Yicaris*, which they classified as a crown-group crustacean, and Siveter et al. (2007b) described the Silurian *Tanazios* which they interpreted as a stem-lineage crustacean. All post-mandibular limbs of *Tanazios* are biramous with two slender, blade-like, tapering exites on the outer margin of the protopodite (Fig. 11.8a), which were identified as epipodites by Siveter et al. (2007b). Boxshall (2007) considered that *Tanazios* should probably be classified as a member of the Labrophora but noted that the presence of two epipodites could be interpreted as evidence that such a state was basic to the Eucrustacea ground plan.

In *Yicaris,* three exites are present along the lateral margin of the protopodal part of the post-mandibular limbs. They were homologized with the epipodite plus pre-epipodite of anostracan Branchiopoda, and a ground plan of three epipodites per limb was suggested for the Eucrustacea (Zhang et al. 2007) or the Eubranchiopoda (Maas et al. 2009). Boxshall (2007) considered that the pattern of development in *Yicaris* (Fig. 11.10a–c) was significantly different from that of branchiopodan epipodites and regarded the evidence supporting the inference that these structures were homologues of the crustacean epipodite plus two pre-epipodites as weak. Boxshall and Jaume (2009) subsequently pointed to differences in form and in the timing of the appearance of the epipodite and pre-epipodite anlagen in anostracan embryos (Møller et al. 2004) and of the exites in *Yicaris* and inferred that the structures in the latter represent an independently derived exite series. Maas et al. (2009) reconsidered the evidence from the Cambrian fossils and concluded that the three exites were present in the ground pattern of their Entomostraca and that these were retained in *Yicaris* and in the Branchiopoda.

The timing of appearance of these structures during development is very different (cf. Figs. 11.7, 11.10). In Branchiopoda, the epipodite (and pre-epipodite) appears very early when the limb primordium comprises a simple transverse ridge of tissue subdivided by slight indentations on the free margin (Fig. 11.7a). As this limb develops, the lobes (presumptive endites, rami, epipodite and pre-epipodite) become better defined (Fig. 11.7b), so by the time the developing limb swings from a transverse to a dorsoventral orientation, the epipodite is already clearly differentiated. In contrast, in *Yicaris* (Fig. 11.10a–c), the bilobate limb bud has a dorsoventral orientation (Fig. 11.10a) before any rudiment of any outer lobe appears (Fig. 11.10b). Assuming the posterior to anterior limb series serves as a surrogate for the development process in *Yicaris*, the three exites appear sequentially, together with the setation elements of the rami and the endites. The development of these exites on the outer margin of the protopodite of *Yicaris* has much in common with the sequential appearance of setation elements and raises doubts as to their homology with the epipodite and two pre-epipodites of the Branchiopoda.

11.4 Heteronomy of Post-antennulary Limbs

A corollary of the hypothesis that the arthropodan ground plan included only two limb types (a multi-segmented single-axis antennule and a biramous post-antennulary limb) is that the post-antennulary limbs formed an essentially homonomous series with little or no differentiation along the A-P axis except in relative size. This describes the trilobite condition: *Phacops,* for example, has paired antennules followed by a homonomous series of post-antennulary limbs (Bruton and Haas 1999). Other Cambrian arthropods, such as the xandarellid *Cindarella*, similarly show a homonomous series of post-antennulary limbs

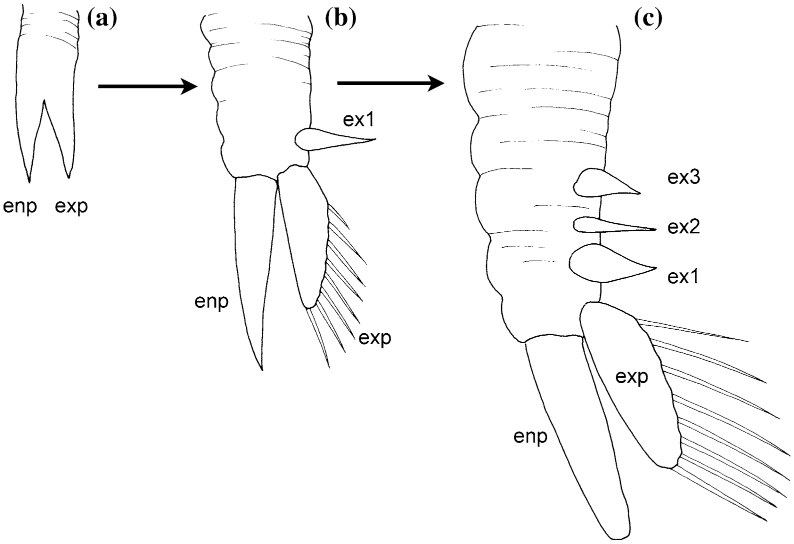

Fig. 11.10 Simplified schematic showing development of exites on trunk limbs of *Yicaris*. **a** Early biramous limb bud in dorsoventral orientation showing endopodite (*enp*) and exopodite (*exp*). **b** More anterior limb with distalmost exite present on protopodite of limb. **c** More anterior limb with three exites present on protopodite (redrawn from data in Zhang et al. (2007) and Maas et al. (2009))

without significant A-P differentiation (Ramsköld et al. 1997).

Most arthropods exhibit some degree of cephalization in which one or more pairs of post-antennulary limbs are modified as specialized feeding appendages. A few Palaeozoic arthropods, such as *Marrella* and *Ercaia,* have just one pair of post-antennulary limbs differentiated from the posterior members of the series (Whittington 1971; Chen et al. 2001). Other fossil arthropods have the anterior two, three or more pairs of post-antennulary limbs differentiated. Mandibulates exhibit heteronomy of the post-antennulary limbs, as exemplified by the naupliar and the post-naupliar limbs in crustaceans. The naupliar limb series comprises the uniramous antennules plus the biramous antennae and mandibles: they differ markedly from the post-naupliar limbs (maxillules, maxillae and trunk limbs) which form a basically homonomous series. This progressive cephalization is the dominant processes underlying the trend towards increasing diversity of arthropodan limb types in the Palaeozoic (see Boxshall 2004). However, Boxshall (2004) also recognized that a basic biphasic arrangement of the post-antennulary limbs into two homonomous series (anterior and posterior) is clearly expressed in early cheliceromorphs. In Palaeozoic forms, it is little modified by specialization within either block. The differences between this biphasic model and the cephalization model presumably reflect differences between the *Hox* genes control mechanisms of the cheliceromorphs and the mandibulates.

Specialization in limb structure along the A-P axis commences with the first post-antennulary limb. The subsequent process of cephalization is progressive, involving the differentiation of increasing numbers of limb pairs in different lineages. Cephalization in all extant arthropod lineages is under the control of *Hox* genes which specify the identity of segments along the A-P axis of arthropods and, thus, play a major role in determining limb morphology. The basic set of *Hox* genes common to extant members of the four major groups of Arthropoda comprises the following genes (orthologues of the *Drosophila* genes): *labial* (*lab*), *proboscipedia* (*pb*), *Hox*3, *Deformed* (*Dfd*), *Sex combs reduced* (*Scr*), *Antennapedia* (*Antp*), *Ultrabithorax* (*Ubx*), *abdominal A* (*abdA*) and *Abdominal B* (*AbdB*)

(Averof et al. 2010). The different *Hox* genes are expressed in different regions along the A-P axis of the body and comparative analysis of these expression patterns suggests that changes in regulation of *Hox* gene expression are correlated with segmental specialization and tagmosis in all arthropods (Akam et al. 1988; Damen et al. 1998; Telford and Thomas 1998; Abzhanov et al. 1999; Hughes and Kaufman 2002). The differences between limbs along the A-P axis reflect the functioning of *Hox* genes acting as selectors. For example, in *Drosophila,* specification of the antenna corresponds to the absence of *Hox* gene input; and thoracic leg identities reflect the action of a single gene: *Scr* for the first legs, *Antp* for the second legs and *Ubx* for the third (Struhl 1982).

Differences in fine-tuning of expression patterns can also occur within, as well as between, major arthropod taxa. Changes in expression of *pb* in the hemipteran *Oncopeltus fasciatus*, together with changes in function of the genes *Dfd*, *Dll* and *cap "n" collar* (*cnc*), correlated strongly with the evolutionary transformation of the haustellate or sucking type of labium from the more plesiomorphic limb found in orthopterans (Rogers et al. 2002). This transformation is slightly different in *Drosophila* where specification of the development of the labial imaginal disc yielding the adult proboscis involves the joint action of both *pb* and *Scr* (Percival-Smith et al. 1997; Joulia et al. 2006).

Changes in the function of *Hox* genes are correlated with changes in segmental organization or tagmosis (Averof and Patel 1997) and have probably played a key role in generating the diversity of arthropod limbs (Liubicich et al. 2009). *Ubx* provides one of the clearest examples. Shifts in the anterior boundary of *Ubx* expression are correlated with functional shifts in morphology within crustaceans (Averof and Patel 1997; Scholtz et al. 2008; Averof et al. 2010). The correlation between the anterior expression boundary of *Ubx* and the position and number of pairs of maxillipeds in crustaceans is striking, and knock-down methods have now been used to reduce *Ubx* expression in the model amphipod *Parhyale* resulting in transformation of walking legs to a maxilliped-like identity (Liubicich et al. 2009).

In insects, the anterior boundary of *Ubx* expression lies in the third thoracic segment and expression extends back through most of the abdomen. *Ubx* expression is instrumental in specifying the boundary between thorax and abdomen, regulating segmental identities and repressing leg development on abdominal segments by repressing *Dll* (Angelini et al. 2005). In addition to this role in A-P axis patterning, *Ubx* also regulates other aspects of development of the third leg, such as the size of the enlarged jumping legs of orthopterans (Mahfooz et al. 2007). In myriapods *UbdA* (combined *Ultrabithorax* and *abdominal A*), expression starts in the second trunk segment and correlates with the morphological differences between the first and second trunk limbs (Grenier et al. 1997). The anterior boundary of *Ubx* expression starts in the second opisthosomal segment in chelicerates (Popadić and Nagy 2001) irrespective of the differences in the morphology of the anterior opisthosoma between spiders, scorpions and the xiphosuran *Limulus*. However, later in development, the anterior boundary of expression of *UbdA* in *Limulus* moves forward one segment to the first opisthosomal segment bearing the chilaria. In chelicerates, therefore, changes in morphology of the first opisthosomal segment are either not associated with changes in *UbdA* expression or correlate only with later changes in *UbdA* expression.

The basic *Hox* gene set is shared by all arthropods and was present in the common lobopodian/arthropodan ancestor, yet the ancestor of the arthropods is hypothesized as possessing a homonomous series of post-antennulary trunk limbs. The original role of some of these *Hox* genes seems obscure with respect to limb differentiation, in an ancestral form with an undifferentiated, homonomous limb series behind the antennules. Given the primitive lack of differentiation along the limb series and the different pathways towards tagmosis exhibited across the Arthropoda, it seems likely that this will be reflected in a diversity of roles for *Hox* genes across arthropod lineages.

11.5 Conclusions

Modern arthropod phenotypes display an amazing diversity of limb types and their limbs are carried on segmented bodies that are patterned along their A-P axis by a basic set of *Hox* genes common to all four major groups of extant Arthropoda. These *Hox* genes play a pivotal role in specifying limb identity, regulating the cascade of genes that are responsible for patterning the limb itself. The early establishment of the P-D axis by the leg gap genes is also a general feature of limb patterning in the development of all arthropods, as is the Notch signalling pathway which is pivotal in the process of subdivision along the P-D axis. The mechanisms responsible for regulating subdivision of the P-D axis into segments or into annuli seem to diverge downstream of the Notch signalling pathway. Levels of Notch activity are central to the fine-scale regulation of joint production and the Notch signalling pathway is also involved in the specification of muscle founder cells. The domains established by the leg gap genes and the operation of the Notch signalling pathway within those domains appears to provide the basic P-D location information for all downstream processes that take place within the limb, including joint formation, muscle and tendon formation and attachment, endite formation and setal patterning.

Less is known about the patterning of the arthropod exopodite, but it seems likely that the same basic mechanism will regulate the P-D subdivision of both rami. New data emerging from fossil marrellomorphs and cheliceromorphs demonstrate that exopodal form was much more variable in early Palaeozoic arthropods than hitherto realized. The arthropodan exopodite exhibits significant morphological variation from multi-segmented to flagellate, and from cylindrical to foliaceous and it would benefit from more focused study.

Comparative data from different arthropod taxa show that homologous patterning domains do not necessarily mark homologous morphological domains. At present, it seems unlikely therefore that gene expression patterns will provide us with reference points allowing the identification of homologies between the component segments of chelicerate, insect, myriapod and crustacean walking limbs. However, a possible exception might be limb components with very specific functional attributes that are reflected in cellular physiology. The epipodites of the branchiopodan trunk limb and malacostracan pereopod, for example, express several genes that are not expressed elsewhere; presumably, these are linked to specific cellular functions related to osmoregulation and gaseous exchange roles of the epipodite epithelial cells.

There remains a major gap in our knowledge—the gap between the new paradigm emerging from developmental genetics and the morphological study of phenotypes. In time, this gap will be filled by cell fate studies and clonal composition analysis and should transform our ability to understand the development of arthropod limbs through the entire timeline from specification to adult phenotype.

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