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Insect–crustacean relationships: insights from comparative developmental and molecular studies

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SUMMARY

The phylogenetic relationships between the major arthropod groups are still far from being resolved. Phylogenetic analyses have usually relied on detailed morphological comparisons which are confounded by the extensive occurrence of convergence. We examine the available morphological evidence in the light of recent comparative developmental and molecular studies and suggest ways in which genetic–developmental information could help assess homology and overcome the problem of convergence. On the basis of such considerations we support the common origin of crustaceans and insects from a crustacean-like mandibulate ancestor. Focusing on the specific relationships between crustaceans, myriapods and insects, we suggest that insects could emerge from this crustacean-like ancestor independently from myriapods, and after the major crustacean radiations.

1. INTRODUCTION

Although the major taxonomic groups that have been described within the arthropods (the insects, myriapods, crustaceans, chelicerates and trilobites) appear to be firmly established and cohesive, the phylogenetic relationships between them are still a matter of intense controversy. Indeed, the very existence of the Arthropoda as a true monophyletic taxon has been seriously questioned. In this review we examine briefly some of the major arguments in arthropod phylogenetics in the light of new evidence from the fields of developmental genetics and molecular phylogenetics. It is only recently that a molecular–developmental approach to evolutionary problems has become possible. Not surprisingly, many of the arguments presented in this paper are still incomplete. We believe, however, that bringing the available molecular–developmental information within the evolutionary debate (and vice versa) can already prove fruitful. We focus specifically on the question of insect–crustacean relationships; at present this seems to be particularly suited for comparative developmental study.

(*a*) *Background to arthropod phylogenetics*

Arthropods are thought to derive from a segmented protostome lineage that arose early in the evolution of the triploblastic metazoans (Adoutte & Philippe 1993; Conway Morris 1993). Traditionally they have been viewed as a well-defined monophyletic group (see Lankester 1904; Carpenter 1906; Snodgrass 1952), united by striking similarities in segmental organization, articulating jointed limbs, exoskeletal structures, moulting, internal organs, coelomic cavities, eyes, etc. This view, however, has been challenged by Manton, Anderson and Tiegs, on the basis of 'irreconcilable' differences in the functional morphology of mandibles, limbs and early embryogenesis (Tiegs & Manton 1958; Anderson 1973, 1979; Manton 1977). These authors have suggested a polyphyletic origin, with at least three independent arthropod lineages arising separately within the protostome animal clade from non-arthropod ancestors (see figure 1*a*). They discount all shared similarities as extensive convergences arising from the adaptive requirements of a hardened exoskeleton (see Willmer 1990, ch. 11).

Many conflicting phylogenetic arrangements have been proposed for the relationships between the major arthropod groups (the main alternatives are summarized in figure 1). The status of the lobopods has been important in these considerations (Manton 1977; Budd 1993; Fortey & Thomas 1993). The lobopods, which include modern-day onychophorans, are a group of segmented 'arthropod-like' creatures with a long fossil record but uncertain phylogenetic affinities. Trilobites, chelicerates, crustaceans and lobopods, already established and distinct in Cambrian faunas, are thought to represent early offshoots in the 'arthropod' radiation. Myriapods and insects appear later in the fossil record – during the Silurian and Devonian, respectively (Robison & Kaesler 1987; Jeram *et al.* 1990; Shear 1990, 1991) – and are generally considered as a monophyletic lineage (variably named as tracheates, atelocerates or Antennata) arising from one of these pre-existing groups. Two competing views have been proposed for the origins of the insect–myriapod clade. One considers the origin of 'whole-limb' jaws and uniramous limbs as particularly informative and suggests emergence from a lobopod ancestor (the uniramian clade, see figure 1*a*; Manton 1977; Willmer 1990; Schram 1991). The other places weight on a considerable number of presumed synapomorphies, including detailed similarities in head segmentation, eyes, mandibles and limbs, to suggest the origin of insects from crustacean-like ancestors (the

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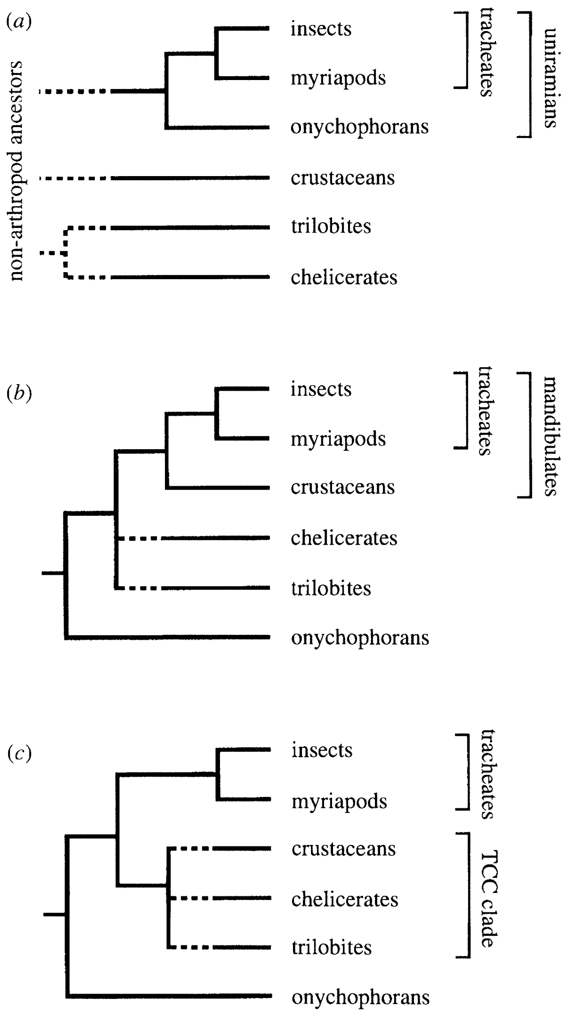

Figure 1. Phylogenetic relationships of main arthropod groups. The three main alternative hypotheses are represented: (*a*) the polyphyletic-uniramian tree; (*b*) the 'mandibulate' tree; and (*c*) the 'TCC' tree. The branch lengths are arbitrary.

mandibulate clade, see figure 1*b*; Lankester 1904; Carpenter 1906; Snodgrass 1938, 1951; Sharov 1966; Boudreaux 1979; Paulus 1979; Weygoldt 1979; Ax 1987; Brusca & Brusca 1990, p. 691; Kukalová-Peck 1992; Wägele 1993). The crustaceans appear to occupy a pivotal position in these schemes, in some cases being allied to the myriapods and insects (the mandibulate clade, see earlier) and in others to the chelicerates and trilobites (the 'TCC' clade, see figure 1*c*; Cisne 1974*b*, Hessler & Newman 1975; Schram & Emerson 1991; Briggs *et al.* 1992, 1993). They are often represented at a very basal node in arthropod phylogeny and sometimes even depicted as paraphyletic with respect to other arthropod groups (see Briggs & Fortey 1989; Della Cave & Simonetta 1991; Briggs *et al.* 1993).

The fossil record indicates that a great diversity of forms was produced very early in the evolution of the arthropods, in a striking radiation which occurred during the early Cambrian, approximately 560 million years ago (Whittington 1985; Briggs & Conway Morris 1986; Valentine & Erwin 1987; Conway Morris 1989; Briggs & Fortey 1989; Della Cave & Simonetta 1991). Although the lineages surviving to the present day reflect only a subset of those already established during the Cambrian explosion, there is evidence to suggest that overall morphological diversity has been sustained by subsequent adaptive radiations (Briggs *et al.* 1992, 1993; Wills *et al.* 1994); the surviving body plans seem to have been increasingly elaborated through the functional diversification of individual segments and appendages (Lankester 1904; Cisne 1974*a*; Flessa *et al.* 1975). It is, therefore, generally thought that the 'explosive' radiation early in the history of arthropods was followed by a long period of adaptive, and often convergent, evolution.

Because most phylogenetic reconstructions rely on direct morphological comparisons and fossil evidence, the origins of major arthropod groups are likely to be obscured by subsequent adaptive radiations. For example, similarity in the branching structure of limbs, patterns of segmental diversification, respiratory systems and developmental modes have all been considered as particularly important phylogenetic indicators, yet these characters have almost certainly been subject to extensive morphological convergence. To assess the evolutionary origin of such adaptive phenotypic traits seems to be a central problem for phylogenetics.

(*b*) *Approaches to phylogenetic reconstruction: getting around the problem of convergence*

There are a number of approaches that could minimize this problem of convergence. One possibility is to base phylogenies on characters that are adaptively 'neutral' and therefore unlikely to converge to a particular adaptive outcome. Molecular phylogenetics claim to do exactly this: at the molecular sequence level, the vast majority of observable changes appear to be adaptively neutral (Kimura 1983). Another alternative is to consider features whose likelihood of occurring is so low that we would not expect them to appear more than once, even under strong directional selection. Such unique evolutionary novelties should provide reliable phylogenetic markers for deep evolutionary radiations. The structure and composition of the *Hox* gene clusters may provide such markers; for example, the duplication and diversification of *Ubx* and *abdA* from a common gene precursor is likely to be a unique synapomorphy of a particular phylogenetic group (see Akam *et al.* 1994).

A third approach, one which we examine in more detail here, is to utilize conventional morphological or developmental characters, but to complement these with molecular information in order to define more reliably the homologies underlying the apparent character states. This approach is based on the assumption that systems with a common evolutionary descent, however modified, are likely to retain some constrained aspects of their ancestral generative system (for a discussion of the concept of homology in relation to continuity of information see Wagner 1989). In contrast, convergent phenotypic traits, however similar, would be unlikely to converge through identical genetic and developmental changes, especially when generative rules are sufficiently complex to allow multiple independent ways for achieving similar effects.

The stability of conserved genetic and developmental pathways could therefore provide a tool for the assessment of homology. The common ancestry that underlies homologous structures and processes should be reflected, at least to some extent, in the conserved activity (patterns of expression, participation in specific pathways) of particular gene products. These can be used as markets to examine the homology and history of morphological transitions.

In recent years we have seen great improvement in our understanding of how genetic information relates to the development of morphological features. Much of this knowledge comes from detailed studies on a small number of species, like the fruitfly *Drosophila melanogaster* (see Bate & Martinez-Arias 1993), which are particularly suited for genetic and developmental work. Based on the detailed background information gained from such 'model systems', and using a comparative approach, it is becoming possible to examine change or conservation of developmental mechanisms in other organisms. Within the arthropods, insects are the obvious point of departure for such comparative studies; they are the source of all our knowledge on a whole range of developmental problems. Crustaceans, on the other hand, have been particularly interesting for comparison because they occupy a pivotal phylogenetic position, display extensive morphological diversity and probably share a common segmental origin with insects. This view is now strongly supported by detailed comparative studies (Dohle & Scholtz 1988; Patel *et al.* 1989*a*). Comparisons with insects can therefore be founded on a common well-defined morphological framework. Although clearly relevant to the phylogenetic problem, comparisons with other arthropod groups (e.g. myriapods, chelicerates) have lagged behind.

It may be that information on molecular structure alone will one day resolve arthropod relationships; the metazoan genome can provide a vast number of characters for phylogenetic reconstructions, many of which have very different evolutionary properties (complexity, evolutionary plasticity, rates of evolution). However, no matter how successful the molecular approach might be in reconstructing phylogenetic relationships, our knowledge of the arthropod radiation will remain incomplete without a clear picture of the evolution of arthropods themselves (structural homologies, morphological transitions, adaptive strategies). To achieve this, criteria will have to be established for a rigorous assessment of homology in morphological characters.

To date, such assessments have been made by rather arbitrary criteria, based almost exclusively on external morphological appearance. We believe that the establishment of positional or structural homologies on the basis of conserved genetic information and underlying developmental mechanisms will provide a useful complement to the existing comparative traditions and lead to a more profound understanding of the concept of homology itself. As specific questions come into sharper focus and the relevant molecular markers are defined, the comparative genetic–developmental approach will provide important insights into the history, mechanisms and constraints of morphological transitions.

2. THE LAST COMMON ANCESTOR OF CRUSTACEANS AND INSECTS

Varied opinions on the phylogenetic relationships of crustaceans and insects have led to differing views as to the nature of their last common ancestor. The monophyletic view claims that the ancestor was a crustacean-like arthropod, whereas the polyphyletic view implies it must have been a more distant protostome form. In this section we examine the different phylogenetic hypotheses on the ancestry of crustaceans and insects, indicate how these relate to recent genetic-developmental observations, and suggest, where possible, how these might be addressed within the present framework of comparative developmental studies.

(*a*) *Distant protostome ancestor* (*polyphyletic view*)

The polyphyletic view has been supported principally on the basis of three key characteristics: the structure of mandibles, limbs, and early embryonic fate maps (Tiegs & Manton 1958; Anderson 1973, 1979; Manton 1977; Willmer 1990, ch. 11).

(i) *Mandibles*

Manton has argued that insect and myriapod mandibles are homologous to those of Onychophora, and so fundamentally different from those of the crustaceans that they could not be commonly derived (Manton 1977). Specifically, it was argued that the biting surfaces in 'uniramian' (onychophoran, myriapod and hexapod) mandibles represent the tip of the entire limb ('whole-limb' jaws), unlike crustacean mandibles where the biting surfaces are modified coxal endites of the limb ('gnathobasic' jaws). However, the argument that all uniramians have 'whole-limb' jaws has been repeatedly challenged by: (i) the comparative anatomy of the jaw musculature, showing detailed similarities between insect and crustacean jaws (Snodgrass 1950; Boudreaux 1979); (ii) the putative homology of the mandibular segment in insects and crustaceans, but not in onychophorans (Snodgrass 1951; Weygoldt 1979); and (iii) a proposed morphological groundplan for hexapod limbs, based on fossil evidence, which refutes the idea of the insect 'whole-limb' jaw (Kukalová-Peck 1991, 1992).

Relevant genetic-developmental evidence comes from the expression of the *Distal-less* gene, a marker and possible determinant of the distal portion of the limbs in insects (Cohen & Jürgens 1989). *Distal-less* is expressed in all appendages except mandibles (including thoracic legs, abdominal prolegs, maxilla, labium and antenna; Panganiban *et al.* 1994). This strongly suggests that the insect mandible must lack the distal portions of the limb, providing further evidence to the view that it is fundamentally similar, and probable homologous, to the crustacean gnathobasic jaw. Additional markers for different proximodistal positions of the limb should soon be available for a more detailed comparative investigation (see Cohen 1993).

(ii) *Limbs*

The distinction between various types of branched and unbranched limb structures has received much phylogenetic attention (e.g. Manton 1977; Emerson & Schram 1990; Budd 1993). According to Manton, the morphological differences that separate the different types of uniramous and biramous limbs are so fundamental that any transformation between them would be functionally implausible (Manton 1977). Other authors have argued, however, that all arthropod limbs can be characterized by a common homologous groundplan (Snodgrass 1935, 1938; Kukalová-Peck 1991, 1992), and indicated that even the conventionally 'uniramous' insect and myriapod limbs show vestiges of an ancestral biramous-polyramous state, e.g. exites in thoracic limbs of machilids, 'polyramous' limbs in fossil insects, branched pauropod antennae (see Wigglesworth 1973; Kukalová-Peck 1991, 1992).

Although it is clear that different limb types are differentially distributed among the various arthropod groups, the structure of the limbs seems to be more closely associated with aquatic–terrestrial adaptations than strictly with phylogenetic position; terrestrial forms of all lineages (e.g. oniscoid isopods, arachnids) show a strong tendency to uniramy. Such adaptive morphological convergence indicates considerable evolutionary plasticity in altering the branching structures of the limb. In developmental terms, it is likely that such transformations could be achieved by relatively simple genetic changes (e.g. Meinhardt 1983; Struhl & Basler 1993; Basler & Struhl 1994; Diaz-Benjumea & Cohen 1994). Thus we could envisage the transition of an ancestral biramous–polyramous limb to a uniramous state being fixed, early in the evolution of the tracheate lineage, in response to adaptive requirements of the terrestrial habit. The radical phenotypic transformations required by Manton's arguments may not be as implausible as they were previously thought.

Molecular–developmental markers for limb pattern and position provide additional information to evaluate the history and relations of limb structures. Using clonal restrictions and *engrailed* expression as reference markers, the position of the limbs has been mapped to homologous positions within insect and crustacean segments (Dohle & Scholtz 1988; Patel *et al.* 1989*a*; Scholtz 1992). The recent proposal that biramy could reflect diplosegmental fusion of adjacent segments (the 'duplosegmentation hypothesis'; Emerson & Schram 1990; Grosberg 1990; Schram & Emerson 1991) cannot be substantiated by any of the available developmental information (Patel *et al.* 1989*a*; Scholtz *et al.* 1994; Scholtz 1994; Zrzavy & Stys 1994). The similarities observed in the development of insect legs and wings (common embryonic origin of their embryonic primordia (Cohen *et al.* 1993), and similarities in patterning mechanisms (Basler & Struhl 1994)) may help evaluate the hypothesized origin of wings from an ancestral outer limb branch (Wigglesworth 1973; Kukalová-Peck 1983, 1991, 1992).

(iii) *Fate maps*

Anderson carried out an extensive survey of early embryogenesis in various arthropod groups (Anderson 1973, 1979), and suggested that early crustacean development differs radically from that of insects, myriapods, onychophorans and annelids. In particular, the positions of the mesoderm and the presumptive midgut in early embryonic fate maps appear to be reversed. Anderson concluded that crustaceans must have evolved independently from a more distant protostome group and suggested that crustacean segmentation may be independently derived.

The more extreme interpretations of Anderson's data seem unlikely, as the molecular–developmental evidence for common mechanisms of segmentation in crustaceans and insects is now compelling (Dohle & Scholtz 1988; Patel *et al.* 1989*a*). Several authors have questioned the phylogenetic significance of early developmental events and fate maps (e.g. Weygoldt 1979), pointing to their extreme variability even within tight phylogenetic groups and their association with particular adaptive circumstances. In well-documented cases, 'alternative' early developmental modes appear to have arisen independently several times in closely related organisms and are thought to result from relatively simple genetic alterations (e.g. Sander 1983; Dohle & Scholtz 1988; Walker 1990; Wray & Raff 1991*a*, *b*; Wray 1992; Jeffery & Swalla 1992; Schneider *et al.* 1992; Patel *et al.* 1994).

More generally, the polyphyletic arguments of Manton and Anderson have been criticized for their unusual methodology (see Patterson 1978; Boudreaux 1979; Weygoldt 1979; Brusca & Brusca 1990, ch. 19): they rely on specific character differences to deconstruct the existing arthropod phylogenetic schemes, but fail to propose synapomorphies for any testable alternative phylogeny. More importantly, their insistence on 'irreconcilable' structural differences and functionally 'implausible' intermediate forms seems to be confronted by the observed evolutionary plasticity of these features and the possibility of saltatory morphological transitions. The potential for discontinuity in morphological change is supported by the presence of discontinuous variation within naturally occurring populations (Bateson 1894) and the observation that relatively few or simple changes in genetic-developmental networks can have drastic, intrinsically discontinuous effects in experimental (see Alberch 1989) and natural situations (see Sternberg & Horvitz 1981, 1982; Freeman & Lundelius 1982; Doebley 1992; Ford & Gottlieb 1992; Dorweiler *et al.* 1993).

(*b*) *Arthropod ancestor* (*monophyletic, mandibulate view*)

The more traditional view, that crustaceans and insects have arisen from a common ancestor that was an arthropod itself, has been supported by a considerable number of presumed synapomorphies (see Lankester 1904; Carpenter 1906; Snodgrass 1938; Sharov 1966;

Wägele 1993). We focus on a number of points which seem to be immediately relevant and testable.

(i) *'Arthropodization'*

The cuticular structure and moulting mechanisms are very similar in both groups. Morphological evidence, including the detailed structure, musculature and articulation of the limbs and segments (Snodgrass 1935, 1938; Boudreaux 1979; Kukalová-Peck 1991, 1992), suggests that metameric organization is homologous in all the arthropods. The developmental evidence is now compelling: molecular markers (the expression of the segmentation gene *engrailed*) and clonal boundaries suggest that the 'parasegmental' nature of metameric units and the origin of limbs within these units are shared by both insects and crustaceans (Dohle & Scholtz 1988; Patel *et al.* 1989*a*; Scholtz 1992, 1994; Scholtz *et al.* 1993, 1994, Manzanares *et al.* 1993; Patel 1994), but are significantly different in the annelids, at least in leeches (see Shankland 1991; Lans *et al.* 1993). The relevant evidence is not yet available for other arthropod groups.

(ii) *Segmental structure of the head*

Homology in the segmental structure of the head, including the conserved position and functional specializations of appendages, has been considered as one of the principal synapomorphies that define the mandibulate clade (Snodgrass 1951; Weygoldt 1979; Kukalová-Peck 1992; Scholtz 1994).

The structure of the most anterior portion of the head has been the subject of a long controversy, which has so far been intractable to both morphological, embryological and molecular analyses (see Rempel 1975; Diederich *et al.* 1991; Schmidt-Ott & Technau 1992; Schmidt-Ott *et al.* 1994*a*; Scholtz 1994); it may consist of largely reduced or fused non-segmental (acronal) and segmental derivatives (of a so-called preantennular, protocerebral, ocular or clypeolabral segment). This disputed region, however, is followed by a well-characterized and conserved series of segments in both crustaceans and tracheates, consisting of two pre-oral and three post-oral (gnathal) segments: (i) the first antennal or deuterocerebral segment, which carries the crustacean antennule and the insect–myriapod antenna; (ii) the second antennal, intercalary or tritocerebral segment, which carries the crustacean antenna, but has lost its appendages in myriapods and insects; (iii) the mandibular segment, carrying a gnathobasic mandible in crustaceans, myriapods and insects (see earlier); (iv) the first maxillary segment, with the crustacean maxillule and insect-myriapod maxilla; and (v) the second maxillary or labial segment, carrying the maxilla in crustaceans and labium in insects and most myriapods. The absence of a distinct second maxillary identity in some crustaceans, notably cephalocarids (Sanders 1963), has been variably interpreted as a secondary loss, or as evidence for the independent specialization of this segment within the crustacean and tracheate lineages.

Recent molecular evidence, based on the expression of the segmentation gene *engrailed*, has confirmed this conserved segmental arrangement of the head in both crustaceans and insects (Patel 1989*a*; Fleig 1994; Schmidt-Ott *et al.* 1994*b*; Scholtz 1994). Genes responsible for specifying the identities of these head segments have now been identified (the Hox genes *lab*, *pb*, *Dfd* and *Scr*; also anterior gap genes like *ems*, *otd*, and *btd*), (see Cohen & Jürgens 1991; Jürgens & Hartenstein 1993) and are known to be ancient and widely conserved (see Holland *et al.* 1992). A comparative study of their expression should provide the markers for a more direct test of the proposed homologies of individual head segments in diverse crustaceans and insects. Furthermore, expression data could provide the means to examine relationships with the chelicerate head, where no convincing homologies are documented.

(iii) *Nervous-sensory systems*

The central nervous system (CNS) in crustaceans, myriapods and insects consists of a tripartite brain (with the protocerebral, deuterocerebral and tritocerebral ganglia), and a double nerve cord with a pair of segmental ganglia and a double connective commissure per segment (see Bullock & Horridge 1965, Part III). Detailed comparisons between crustaceans and insects indicate that various cell types within each neuromere have been strikingly conserved between these groups, in both their identity and relative positions (Thomas *et al.* 1984; Whitington *et al.* 1993). A large number of molecular markers for neuroblast and nerve cell types are now available, which could provide direct genetic evidence for the homology of these patterns (Patel *et al.* 1989*a–c*; Doe 1992).

The structure and innervation of the eyes are also strikingly conserved among crustaceans and insects (Paulus 1979; Osorio & Bacon 1994), particularly in the detailed cellular composition of the ommatidial units of the compound eyes. Recent studies on the molecular determination of ommatidial cell fates in *Drosophila* (see Hafen 1991; Moses 1991; Rubin 1991; Hart *et al.* 1993) are likely to provide molecular markers suitable for a more detailed comparative analysis. The striking similarities observed between crustaceans and insects in the cellular pattern in the eyes and the CNS have not been seen in chelicerates or myriapods (Paulus 1979; Osorio & Bacon 1994, see later).

(iv) *Internal organization*

The true coelom in arthropods is thought to have been greatly reduced or even lost. The main body cavity in all the arthropods has been replaced by an open haemocoel. The circulatory system is open, with a dorsal heart. The gut comprises foregut and hindgut, thought to be ectodermally derived, and endodermal midgut (Clarke 1979). Interesting comparative information could be obtained using conserved molecular markers for specific regions of the gut (e.g. Jürgens & Hartenstein 1993; Reuter 1994; Bronner *et al.* 1994; Kispert *et al.* 1994).

The polyphyletic hypothesis claims that all these shared similarities are merely convergent adaptations to similar functional requirements (see Willmer 1990, ch. 11). This may be true for some features. However, these similarities often extend to details whose adaptive significance would be difficult to imagine (e.g. the precise cellular composition of the ommatidial unit in the eye and the segmental ganglia in the nerve cord) and, where available, molecular markers indicate that these features are specified by commonly derived genetic information.

Although clearly relevant to this debate, molecular developmental information is not yet available for other arthropod groups (myriapods, chelicerates). It is for this reason that we have not used molecular markers as independent characters for phylogenetic reconstruction. Instead, we have considered these within the pre-existing framework of comparative morphology in two very specific ways: first to refute the idea of 'irreconcilable' morphological differences on which most polyphyletic arguments are based (see §2*a*), and second to examine the previously proposed homology in a number of morphological features of crustaceans and insects (see §2*b*).

(*c*) ***Crustacean-like ancestor***

Although many of the arguments presented have not been entirely resolved, we believe that there is now strong evidence to support a close relationship between crustaceans and insects. If so, the most recent common ancestor of crustaceans and insects must have possessed the various synapomorphies that define this clade, most notably the conserved segmental structure of the head and cellular patterns in the nervous system and the eyes (as presented in the previous section). In addition, the tritocerebral appendage (second antenna) and the outer branches of the limbs (exopodites) are generally thought to have been present in that ancestor, and lost secondarily in myriapods and insects. Three lines of evidence point in that direction: (i) vestiges of both these structures may be seen in myriapods and insects (see Tiegs 1940; Wigglesworth 1973; Kukalová-Peck 1992); (ii) the fossil record indicates that crustacean forms were present significantly earlier than myriapods and insects (more than 100 Ma); (iii) adaptation to terrestrial life provides adequate explanation for these changes. Such reduction or complete loss is seen convergently in other terrestrial forms (e.g. reduction of the 1st antenna in oniscoid isopods). Similar selection pressures would eliminate a naupliar stage from the life cycle.

We conclude that all the crustacean synapomorphies may have been shared by the presumed mandibulate ancestor (other characters which are common to many crustaceans, like the carapace and stalked compound eyes, are not diagnostic features of this group).

3. THE RELATIONSHIPS BETWEEN CRUSTACEANS, INSECTS AND MYRIAPODS

(*a*) ***Paraphyletic crustaceans?***

If the common ancestor of the mandibulates was a crustacean itself (see Lankester 1904; Carpenter 1906; Sharov 1966, pp. 55–60), it is an almost inescapable conclusion that modern crustaceans must be paraphyletic with respect to myriapods and insects. The earliest fossil representatives of myriapods and insects appear during the Silurian and Devonian, respectively (Jeram *et al.* 1990; Shear 1990, 1991). But long before that, by the end of the Cambrian, several major crustacean clades were already established and distinct: possibly malacostracans, maxillopods and branchiopods (Briggs 1978; Müller 1979; Robison & Kaesler 1987; Müller & Walossek 1988; Walossek 1993; Butterfield 1994; Briggs *et al.* 1992, 1993; Wills 1994). If these records are a true reflection of the emergence of these groups, then the crustacean ancestors of myriapods and insects must have lived significantly later than the earliest crustacean radiations.

In terms of segmental organization, crustaceans are by far the most diverse group within the arthropods. Compared to the conservative body plan of insects (composed of head, thorax and abdomen with highly conserved numbers of segments), crustaceans display a striking variety of body plans, both in terms of segment numbers, tagmosis and specific segment types (see Cisne 1974*a*; Schram 1986). This unusual diversity, combined with the early appearance of crustaceans in the fossil record, suggest that this group may occupy a very deep position in arthropod phylogeny (Sharov 1966; Briggs & Fortey 1989; Della Cave & Simonetta 1991; Briggs *et al.* 1993).

To demonstrate conclusively the emergence of tracheates from some crustacean form, it may be important to examine whether myriapods or insects are more closely related to some crustacean groups than others. Various morphological affinities have been suggested in the past, e.g. similarities with malacostracans in segment numbers (Carpenter 1906), with anostracans in the structure of the head (Snodgrass 1951), with archimalacostracans (Sharov 1966, pp. 55–60), but convincing synapomorphies have not yet been established. We suspect that such synapomorphies could be found in the segmental composition of the head (see *Canadaspis* and cephalocarids for differences in the number of distinct gnathal segments; Sanders 1963; Briggs 1978), the general organization of the body (body plans, tagmosis) and detailed structural similarities in nervous systems, eyes or other organ systems (the CNS, especially, provides a huge and relatively conserved diversity of cell types which may be phylogenetically informative). Phylogenetic relations could also be reconstructed, independent of morphological considerations, using nucleic acid or protein sequence information (for further details see below). So far this question has not been properly addressed.

(*b*) ***Polyphyletic tracheates?***

The search for the more detailed relationships between crustaceans and insects has been based on the widely held assumption that tracheates (myriapods and insects) are monophyletic. Our arguments for crustacean paraphyly are not strictly dependent on this view; the 'crustacean origin' of insects could have

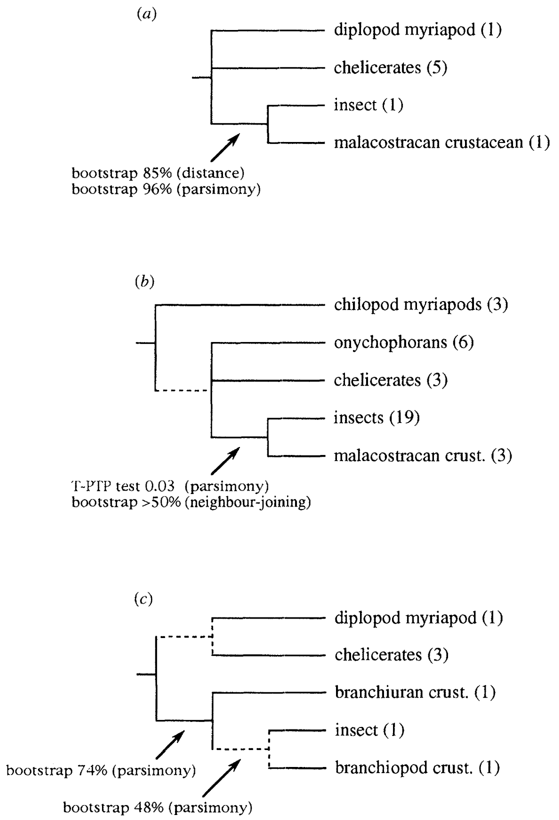

Figure 2. Molecular phylogenies of arthropods. Phylogenies based on: (*a*) 18*S* rRNA (Turbeville *et al.* 1991); (*b*) 12*S* rRNA (Ballard *et al.* 1992); (*c*) 18*S* rRNA (Adoutte & Philippe 1993). The statistical significance of crucial nodes is indicated. Dotted lines indicate nodes which are not statistically significant. The number of species sampled from each group is indicated in brackets. The branch lengths are arbitrary.

occurred indirectly, through a myriapod-like lineage, or directly. Recent results support a very close relationship between crustaceans and insects, but not myriapods, suggesting that the conventional assumption may be misleading.

Principally, this evidence has come from two independent studies of ribosomal RNA sequences (18*S* and 12*S* rRNA; Turbeville *et al.* 1991; Ballard *et al.* 1992; see figure 2*a*, *b*). The phylogenetic trees reconstructed from these data suggest a close sister relationship between crustaceans and insects, with myriapods forming a more distant clade. These results should be treated with caution as only a small number of myriapods have been sampled in each case: one diplopod (Turbeville *et al.* 1991) and three chilopods (Ballard *et al.* 1992). However, the fact that two independent molecules have produced such similar results reinforces the conclusion that the tracheates may not be monophyletic.

This proposal is also supported by comparative work on the cellular structure of sensory and nervous systems. Striking similarities observed between crustaceans and insects in the relative positions and axonal pathways of specific cells within the CNS, suggest a common plan for neurogenesis in these two groups (Thomas *et al.* 1984; Scholtz 1992; Whitington *et al.* 1993). In contrast, the neuronal pattern in myriapods bears no obvious similarities to that found in the insects (Whitington *et al.* 1991, 1993). Similarly, the cellular composition and neural circuitry of the compound eyes appear to be tightly conserved between crustaceans and insects (Paulus 1979; Osorio & Bacon 1994), but not so in the myriapods (however, myriapod eyes may not be particularly informative in this respect since they are thought to be secondarily reduced, simplified, or entirely lost in many cases).

The suggestion that myriapods and insects may represent two independent lines of evolution, with the insects more closely allied to crustacean groups, contradicts conventional arthropod phylogeny in one of its most firmly established points. In the light of this new phylogenetic possibility, we present briefly the principal tracheate synapomorphies that have been considered so important in the past: (i) loss of the second antennal appendage; (ii) loss of the outer branches of the limb (uniramy); (iii) a tracheal respiratory system; (iv) Malpighian tubules, arising as proctodeal evaginations; (v) 'whole-limb' mandibles. Most of these presumed synapomorphies (ii–iv) are clearly adaptations to terrestrial life and, therefore, the possibility of them arising by convergence cannot be ruled out. As described already, uniramy appears to be particularly plastic, arising independently several times in crustacean and chelicerate limbs, and the 'whole-limb' origin of mandibles is probably incorrect (see earlier).

The suggestion that myriapods themselves may be paraphyletic (see Lankester 1904; Tiegs 1947; Dohle 1988) further complicates the situation. Chilopods and symphylans are often placed closest to the insects, with pauropods and diplopods emerging at more distant positions of the tree (these results are mainly based on the segmental composition of the gnathal region and the location of genital segments).

(*c*) *Molecular phylogenies*

So far, molecular phylogenies of arthropods have largely focused on their broad relationship to other metazoan phyla (see Field *et al.* 1988; Lake 1990; Adoutte & Philippe 1993) or detailed relationships within the individual groups (see Kim & Abele 1990; Turbeville *et al.* 1991; Carmean *et al.* 1992). The question of inter-relationships between the main arthropod groups, especially crustaceans, myriapods and insects, has not yet been examined in any systematic way. We believe that to readdress this question conclusively, relationships will have to be examined without prior assumptions as to the monophyly of each group. Possible paraphyletic or polyphyletic relationships must be analysed by using multiple and widely divergent 'representatives' of higher taxa.

As described earlier, two studies using restricted datasets from two independent molecules have so far produced surprisingly congruent results (Turbeville *et al.* 1991; Ballard *et al.* 1992; see figure 2*a*, *b*), supporting a sister-group relationship between crustaceans and insects and the independent origin of myriapods, i.e. tracheate polyphyly. However, the detailed insect–crustacean relationships remain untested; none of these studies have examined the possibility of crustacean paraphyly (in both cases a single crustacean group, the Malacostraca, was represented). In a more recent study of 18*S* rRNA (Adoutte & Philippe 1993) (see figure 2*c*), single representatives of two crustacean groups were shown to be paraphyletic with respect to an insect, but this arrangement was not statistically significant. We have re-examined an enlarged 18*S* rRNA dataset using published sequences from several crustacean groups (branchiopods, malacostracans, ostracods and branchiurans). As in the previous studies, tracheate polyphyly was strongly supported. Insects and crustaceans consistently emerged as a monophyletic assemblage (in almost every case crustaceans were paraphyletic with respect to the insects), but the detailed relationships between the insect and crustacean groups were not significantly resolved (unpublished data). There is clearly much scope for the extension of these studies, especially with regard to more extensive sampling of diverse crustacean and tracheate clades, and the use of other molecules to provide phylogenetic information.

These results cannot yet conclusively resolve the question of insect–crustacean relationships. The contribution of molecular studies at present seems to be limited by the poor sampling of various lineages of crucial phylogenetic importance, especially within crustaceans, myriapods and lower insects. However, they are entirely consistent with the proposed polyphyletic composition of the tracheates, the close evolutionary relationship of insect and crustaceans and, indeed, the paraphyly of crustaceans with respect to the insects. The results indicate that these rather unconventional hypotheses at least deserve further serious consideration.

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