yields 30 protein molecules per million calcite unit cells.
13. The volume of a coherence block is 4000^3 Å^3. The volume of a single calcite unit cell is $\sin 60 \times 5 \times 5 \times 17$ Å^3. Hence, a total of 200×10^6 unit cells in one block are surrounded by ~6000 protein molecules (*12*). If we assume two-dimensional β-pleated sheet dimensions, the coverage (protein/mineral ratio) is of the order of 20% for an average value of the intracrystalline protein concentration.
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The Origin of Crustacean Biramous Appendages and the Evolution of Arthropoda

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The evolution of biramous appendages in crustaceans is central to the debate on the origin of the arthropods. It is proposed that the biramous limb evolved through the basal fusion of adjacent pairs of ancestrally uniramous appendages. As a result, the existing system of homology, in which uniramous and biramous appendages are considered equivalent, may be invalid. Similarly, the homology of individual body segments between uniramians, such as insects and myriapods, and arthropod groups with biramous limbs is also called into question. Two uniramian segments, or a diplosegment, may be homologous to a single body segment in biramous groups.

CENTRAL TO THE DEBATE ON ARthropod phylogeny is the relation of the insects and myriapods with uniramous limbs to the arthropods with biramous limbs such as crustaceans, cheliceriforms, and a wide range of Paleozoic fossils including trilobites. Among the extant groups of biramous arthropods, the crustaceans appear to be less derived than the cheliceriforms (*1*) and may be close to a common ancestor with the uniramians. The relation between Uniramia and Crustacea is critical because these groups share such characters as mandibles and a similar cephalic composition that are interpreted either as evidence of a monophylectic origin (*2*), or as convergent evolution of features in unrelated groups (*3*). However, any attempt to create a phylogeny of the arthropods must deal with the question of the origin of biramous limbs.

Some investigators have suggested that biramous limbs arose from uniramous ones through the gradual evolution of an appendicular structure, such as the stylus of some insect legs, into the exopod of the typical biramous appendage (*4*). However, lack of intermediate forms and the extreme variability of such auxiliary limb structures would seem to argue against this hypothesis. Another approach has been to start with biramous (or polyramous) limbs and delete the outer exopod and the basal accessory epipodites to derive a secondarily uniramous limb (*5*). However, this suggestion not only begs the question of the ancestry of the biramous limb, but also minimizes evidence that the resemblance between primary and secondary uniramous limbs is convergent. The failure of such theories to account satisfactorily for the evolution of biramous limbs has been one of the factors leading some authors to conclude that the arthropods are diphylectic or polyphylectic (*3*). Many investigators, however, argue that the proposition that each arthropod group evolved their numerous shared, derived characters independently strains credulity (*2*).

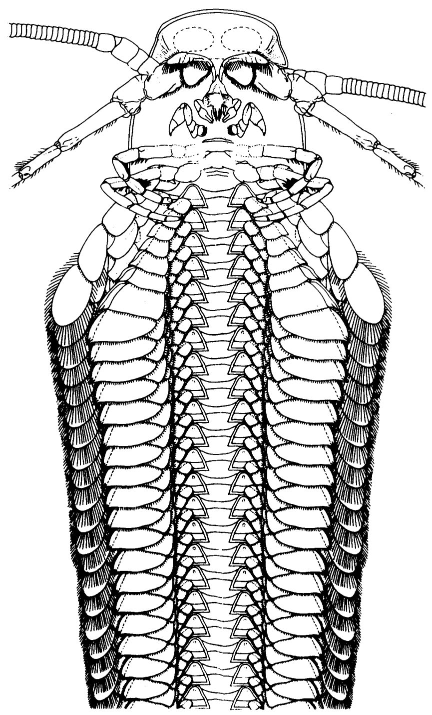

Fig. 1. Ventral reconstruction of *Tesnusocaris goldichi* based on all currently available specimens. The head, with its raptorial postmandibular mouthparts, is v [illegible] remipedes.

Newly discovered fossils of a problematic Mississippian arthropod, *Tesnusocaris goldichi* (*6*), provide anatomical insights that prove crucial to understanding this species (*7, 8*) as well as suggest a new hypothesis for the events of arthropod history. The structure of the head (Fig. 1) confirms a sister group relation of this fossil species to living nectiopodan remipede crustaceans (*9*). The trunk of *Tesnusocaris* also resembles those of extant remipedes in that regionalization of the segments and trunk limbs is absent, but an examination of the locomotory appendages of the fossils reveals a structure quite different from that of Nectiopoda and other crustaceans (Fig. 2C). Instead of the typical, biramous appendages that were expected, we found two distinct sets of uniramous limbs on each segment. There is a midventral series, which may be unique among arthropod limbs in that they appear to be adapted for sculling (*8*). In addition, there is a ventrolateral series of fairly typical swimming limbs, adapted for rowing.

Detailed study of the fossils, as outlined below, suggests that these are two separate sets of uniramous limbs and not the remnants of a biramous limb whose protopodal base has fused into the body wall. (i) The wide separation of the medially situated limbs and laterally positioned appendages on our fossils seems too great to suggest that they were ever associated with a common protopod. (ii) The great differences between the apparent function of the two sets of limbs (*8*) suggest that they evolved separately and that they possessed distinct musculatures. (iii) The first segments of both sets of limbs resemble true coxae seen in various arthropods, rather than the mobile podites typical of the more distal parts of limbs that might be expected if the original coxa had been fused to the body wall. (iv) A comparison of the number of podomeres on the

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fossils to limbs of extant crustaceans reveals that the *Tesnusocaris* trunk appendages have more podomeres than the exopod and endopod of biramian crustaceans. If these limbs had evolved from the secondary loss of podomeres at the base of a biramous limb, fewer podomeres might be expected to result rather than more. Although none of these lines of evidence are conclusive by themselves, together they tend to support a hypothesis that the trunk limbs of *Tesnusocaris* evolved from a diplopodous ancestor (Fig. 2A) and in turn gave rise to the biramous limbs of crustaceans (Fig. 2D) through basal fusion of adjacent pairs of limbs.

Our hypothesis requires a nearly complete revision of the homologies currently used to describe arthropods. The uniramous limbs of animals like *Tesnusocaris* (Fig. 2C) are distinct from, but presumably homologous to, the exopod and endopod of the typical crustacean limb. Because these two sets of limbs exist on a single segment, this condition is quite distinguished from the diplopodous uniramians (Fig. 2A) and cannot be compared to the arrangement seen in the insects and some myriapods (Fig. 2B).

Furthermore, there are other indications that such a fusion of segments may have occurred, that crustacean segments may have been derived from two segments. For example, branchiopods display an extremely primitive form of the typical crustacean central nervous system (*9*). As in all articulates, the primitive condition is a double, ventral nerve cord in the trunk with segmented ganglia. In the branchiopod nerve cord, however, the two widely separated nerve trunks are connected by two nerve commissures in each segment. Various degrees of fusion are seen in the nerve cords of other crustaceans, obliterating this pattern. In contrast, the annelids and most uniramians have only one commissure or ganglion in each body segment; and in the Diplopoda, the double nature of each diplosegment is reflected in two pairs of ganglia. The branchiopod nerve cord with its double commissures appears, possibly, to retain characteristics of a two-segment ancestry.

Another example in this regard is a transitory seventh pair of posterior, abdominal, ganglionic anlagen that appear in the ontogeny of several malacostracans including mysids, stomatopods, tanaids, isopods, and amphipods (*9*). All of these have been interpreted traditionally as indicating the presence of a seventh abdominal segment derived from some ancestor. However, these anlagen may be due simply to the delayed fusion of a second set of ganglia associated with the sixth abdominal double segment. A similar explanation may apply in the anterior abdomen of the mantis shrimp *Oratosquilla oratoria* (*10*) with an anomalous, double arterial supply from the heart to the first abdominal segment.

The closest approximation to the segmental condition of *Tesnusocaris* among living arthropods is found in the more primitive myriapods. The Pauropoda and Diplopoda typically have diplopodous trunk segments—that is, a single segment dorsally is associated with two ventral sternites each with a pair of uniramous limbs. The Scutigeromorpha have both diplosegments and triplosegments, and the Lithobiomorpha have alternating long and short trunk segments that resemble diplopody. The centipedes that pass through several juvenile stages do so by adding two segments at each molt cycle (*11*). Some centipedes, such as the Scolopendromorpha, have only a slight alternation in tergal dimensions, but the Geophilomorpha show no obvious pairing of segments (Fig. 2B). In all the diplopods, there is strong evidence of segmental pairing by multiples of two (*12*). Symphylans have a different sort of pairing of double tergites. Insects show no external evidence of segmental pairing, but the genetic evidence of paired segmental anlagen will be discussed below. The widespread occurrence of segmental pairing among myriapods and insects suggests that this feature may have been shared with their immediate ancestor.

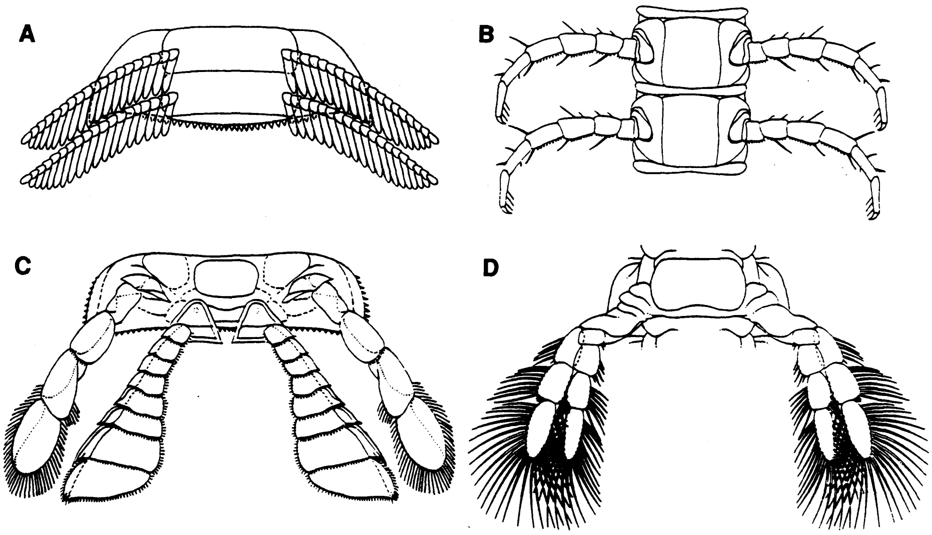

Fig. 2. Semidiagrammatic, ventral views of body segments from representative uniramians and crustaceans. (**A**) Diplosegment of a generalized fossil euthycarcinoidean with uniramous limbs. (**B**) Two segments of a geophilomorph centipede, each with uniramous limbs. (**C**) Segment of the enantiopodan remipede, *Tesnusocaris*. (**D**) Segment of a nectiopodan remipede.

The extant myriapods and insects appear to be much too specialized, however, to have given rise directly to *Tesnusocaris*. The fossil record provides one other group of diplopodous uniramians of interest in this regard, the Euthycarcinoidea (*13*), the details of whose head anatomy are unclear, but appear to have been primitive, with a distinct procephalon bearing the antennae, followed by a short gnathocephalon bearing reduced or absent appendages. The trunk limbs were located typically on diplosegments and triplosegments. The euthycarcinoideans are unique among the uniramians for having trunk limbs consisting of 15 to 24 simple podomeres, each bearing a single, flattened spine in at least one species (Fig. 2A), and a distinct abdomen of four to six limbless segments plus a tailspine. The primitive characteristics of euthycarcinoideans, including a short, poorly consolidated head, a long series of mainly diplopodous, unspecialized trunk appendages with numerous podomeres, and an aquatic habitat, appear to place them near the base of uniramian evolution; they also seem close to a possible ancestor of *Tesnusocaris*.

If segmental patterning in pairs is a unifying feature of arthropods, then evidence for such should be present even in animals that may not, at first glance, appear to exhibit such patterns. Like all insects, *Drosophila* is constructed of distinct segments, and it is not immediately evident that there is any segmental pairing. Nevertheless, although *Drosophila* is an extremely advanced insect, its genetic systems express ontogenetic patterns of segment pairing as postulated from an ancestral body plan.

Such pairing, however, is controlled by several distinct classes of genes (*14*) as is segmental limb development (*15*). Insect segmental expression is influenced by pair-rule genes of two types. Mutations in even-skipped loci result in the deletion of even-numbered segments from the thorax and abdomen, and odd-skipped loci affect oddnumbered segments. The phenotypic expression of insect segments appears to be the result of a balance between two types of pair-rule loci. There are apparently no genes that control the expression of single segments as such. Workers in the field of fruit fly genetics were surprised that the expression of monosegments was the result of the interaction of pair-rule genes. However, it is evident that this segmental pairing is merely another manifestation of the underlying diplomerous organization of uniramian arthropods.

The suggestion that the phenotypic expression of segments could be the result of heterochronies in the segmental duplication cycles (*12*) has important consequences. The question of limb phylogeny shifts from explaining how one morphology evolved into another, to understanding how the timing of duplication cycles affects a range of morphologies. This makes the evolutionary transition of one body plan into another more comprehensible in terms of apparent convergences, character reversals, and discontinuities.

It now appears that uniramians can be more firmly linked with biramous arthropods than ever before. Interestingly, some confirmation of the events postulated here may be emerging from new analyses of molecular data (*16*), wherein uniramians are placed as the first offshoot of a line of evolution leading to the biramous arthropods.

The fact that *Tesnusocaris*, a 310-million-year-old Coal Age animal, provides insights concerning events of arthropod evolution that must have happened 600 million years ago in the early Cambrian is not contradictory. We think that Cambrian fossils do exist that clearly seem to have a similar anatomical form (*8*), but previously have been misinterpreted. *Tesnusocaris* is at present merely the best known fossil with this form. That *Tesnusocaris*, as a missing link in arthropod evolution, is a remipede should not be too surprising. This merely seconds the arguments for remipedes being a sister group to all other crustaceans (*9*). Other investigators hold positions contrary to this view (*5*), although to date no concrete arguments have been put forth against remipedes being built on the most primitive of crustacean body plans.

In conclusion, the assumption of serial homology has been the central paradigm governing comparisons between the body plans of various groups of segmented metazoans. In fact, it would seem impossible to attempt analyses of arthropod body plans without it. In the absence of evidence to the contrary, the paradigm of serial homology seemed not only reasonable, but necessary—despite the fact that comparisons inevitably reveal gross inconsistencies. The paradigm of serial homology is an assumption, however, that is untested and may prove misleading. Now, evidence offers a different possibility.

We suggest that the evolutionary sequence from annelid-like origins, through an onychophoran-like ancestor, to diplopodous myriapod-like uniramians established the base of arthropod evolution from which evolved crustaceans and possibly other biramian groups. It seems that duplication cycles leading to segment pairing and eventual fusion are the unique derived feature of the Arthropoda as a whole and have been a central factor in the flexibility of body plan design that has made the arthropods conspicuously successful since the Cambrian explosion some 600 million years ago.

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Side Chain Contributions to the Stability of Alpha-Helical Structure in Peptides

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Short peptides that contain significant α-helical structure in aqueous solution allow the investigation of the role of amino acid side chains in stabilizing or destabilizing α-helix structure. A host-guest system of soluble synthetic peptides was designed that consisted of chains with the block sequence TyrSerGlu$_4$Lys$_4$X$_3$Glu$_4$Lys$_4$, denoted EXK, in which X represents any "guest" amino acid residue. Circular dichroism spectroscopy indicates that the extent of helicity of these peptides follows the order Ala > Leu > Met > Gln > Ile > Val > Ser > Thr > Asn > Gly. This order differs from both host-guest copolymer values (Met > Ile > Leu > Ala > Gln > Val > Thr > Asn > Ser > Gly) and the tendencies of these amino acids to occur in helices in globular proteins (Ala > Met > Leu > Gln > Ile > Val > Asn, Thr > Ser > Gly), but matches the order found in a series of synthetic coiled-coil α helices, except for Ser. Proton nuclear magnetic resonance analysis of several EXK peptides indicates that these peptides are partially helical, with the helical residues favoring the amino terminus.

ABOUT ONE THIRD OF THE RESIDUES in globular proteins of known structure are estimated to be α helical in conformation (*1*). The reasons for this are imperfectly understood, although the question is of importance in trying to determine or predict how proteins fold. Chou and Fasman (*2, 3*) improved and extended a number of earlier, less complete statistical correlations to estimate the helical propensities of different side chains from the frequency of occurrence of amino acids in helical sequences in proteins of known structure. More current schemes seek to take longer range sequence correlations into account (*4*), but these entail fitting a much larger set of parameters than the simple Chou-Fasman version, comprehensive data for which are not yet available.

Scheraga and his co-workers (*5*) have done experiments to define the quantitative influence of each of the 20 standard side chains on the stability of α helices empirically, introducing the amino acid as a "guest" into a soluble copolymer of the form poly

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