# Origin of the arthropod mandible

SIR — Arthropods, vast in number and with enormous variation in body forms, are a fascinating group. We have found that myriapods (millipedes, centipedes and allies) have different mandibular origins from insects and crustaceans, which is of consequence for resolving phylogenetic relationships among major groups of arthropods.

For more than a century, the phylogenetic relationships among the main arthropod lineages have been a topic of lively discussion. Almost every imaginable combination has been proposed, but at present only two hypotheses are seriously considered: the 'TCC' view, which separates trilobites, crustaceans and chelicerates from the rest of the arthropods[1], and the 'mandibulate' theory, which groups together crustaceans, insects and myriapods[2]. One feature is common to both: the close relationship between myriapods and insects. These two arthropod groups were traditionally united into Atelocerata[3], because they share five adult characteristics: a tracheal system, malpighian tubules, absence of appendages corresponding to the second antennae of crustaceans, unbranched legs, and a mandible (jaw) composed of a whole limb.

The existence of the Atelocerata has recently been questioned by two independent studies reporting molecular data to infer arthropod phylogeny[4,5]. Friedrich and Tautz[4] suggested that crustaceans, and not myriapods, are the sister group of insects, arguing that the first three characteristics common to both myriapods and insects are convergent adaptations to terrestrial life and thus do not reflect a common ancestry. Panganiban *et al.*[6] have shown also that differences between branched and unbranched legs can be caused by a simple developmental switch. In the light of these arguments, it is essential to evaluate critically the fifth feature shared by myriapods and insects, namely, a similarity in the structure of their mandibles.

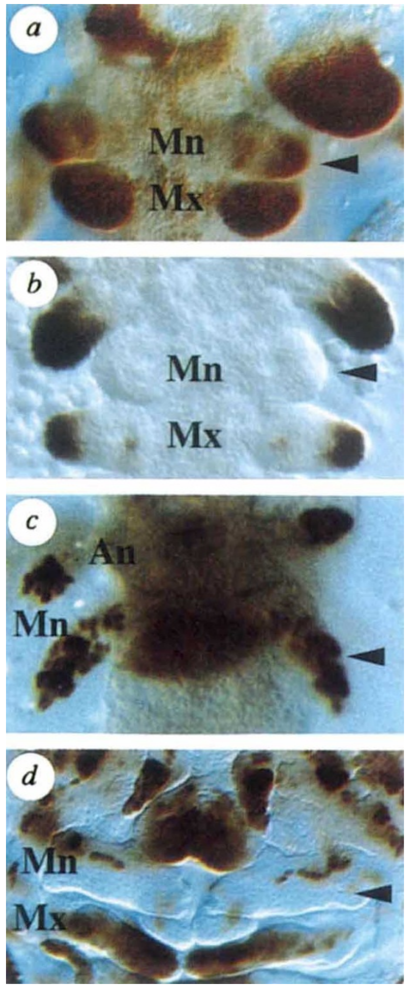
Traditionally, crustacean mandibles are regarded as being formed from a limb base, and ateloceratean mandibles as being composed of a whole limb[7], but this view has been challenged recently[2]. One way to resolve this dispute is to investigate the structure of arthropod mandibles at the molecular level, as suggested by Friedrich and Tautz[4].

We studied the pattern of expression of the homeobox gene *Distal-less* (*Dll*) in the mandibles of the millipede, and compared it with results from insects and crustaceans (*Dll* antibody was kindly provided by G. Panganiban). This gene specifies the distal part of appendages, and therefore can be used as a molecular marker for investigating the structure of the mandibles (whole limb versus limb base only). In the millipede *Oxidus gracilis* (*a* in the figure), *Dll* is expressed in the distal part of the mandibles, as predicted in ref. 4, indicating their whole-limb structure.

In light of the recent discovery of the Cambrian fossil whose head and trunk appendages were long and leg-like[8], the whole-limb mandibles of today's myriapods probably represent an ancestral arthropod state. Thus, we have a testable hypothesis: if myriapods and insects are indeed sister taxa, then *Dll* should also be expressed in insect mandibles. But Panganiban *et al.*[9] have shown that *Dll* is not expressed in mandibles of modern insects. To examine whether the absence of *Dll* is characteristic of the whole insect lineage, we included in our analysis the primitively wingless insect *Thermobia domestica*, and found that *Dll* is not expressed in the mandibles of this species (*b* in the figure); this further suggests that insect mandibles are formed from the limb base and may be similar to the mandibles of adult crustaceans.

Both *O. gracilis* and *T. domestica* undergo direct development, where immature stages differ from the adults mainly in the development of the gonads and genitalia. This allows us to correlate directly embryonic changes in *Dll* expression with structural changes in adult mandibles. In contrast, only crustaceans that undergo larval development have been studied so far[6]. Consequently, the finding that *Dll* is expressed throughout the mandibles of crustacean nauplius larvae (*c* in the figure) is not informative because the larval cells expressing *Dll* do not contribute to the adult structures (as noted in ref. 6). To infer the origins of the mandibles in adult crustaceans, it is necessary to study species that undergo direct development. We therefore included the terrestrial isopod *Armadillidium vulgare*, a direct developer, in our analysis. We found that crustacean mandibles are indeed composed of a limb base only, as is evident by the lack of *Dll* expression in ectoderm (*d* in the figure).

In summary, our data are consistent with earlier predictions[2,4,6] that the arthropod mandible was originally composed of a whole limb and was similar to the present-day mandibles of the myriapods. Further, our data suggest that during arthropod evolution, the mandible structure changed from a whole limb (*a* in the figure) to a limb base only, the latter type being a shared feature between insects and crustaceans (*b*, *d*). This finding has two important implications. First, it argues against the traditional view that insect and crustacean mandibles are fundamentally different[7]; and second, it directly supports the hypothesis that crustaceans, not myriapods, are the sister group of insects[4]. The overall similarity of nervous and visual systems in both insects and crustaceans provides independent support for this hypothesis[10]. These findings lessen the case for uniting insects and myriapods into Atelocerata.




Expression pattern of *Dll* in the mandibular segments of the millipede *Oxidus gracilis* (*a*), the primitively wingless insect *Thermobia domestica* (*b*), the crustacean nauplius larva *Artemia franciscana* (*c*) and the terrestrial isopod *Armadillidium vulgare* (*d*). An, antennal segment; Mn, mandibular segment; Mx, maxillary segment. Arrowheads indicate the mandibular appendages.

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