Fig. 5. Observed and predicted cross-shore bottom elevation profiles spanning a 45-day period. Sea-floor elevation relative to mean sea level observed 1 September 1994, 1900 hours (solid black curve), observed 15 October 1994, 2200 hours (dashed black), and predicted for 15 October 1994, 2200 hours by the energetics (blue) and energetics plus acceleration (red) models versus cross-shore position.

energetics models without acceleration-based transport predicted the offshore migration (*1*, *2*), they had limited skill predicting the total change to the beach over 45 days because they failed to predict onshore migration between storms (*2*). The energetics model that was extended to include acceleration better predicted the change in the sea-floor both onshore and offshore of the bar crest (Fig. 4), and the overall evolution of the cross-shore depth profile (Fig. 5).

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Hexapod Origins: Monophyletic or Paraphyletic?

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Recent morphological and molecular evidence has changed interpretations of arthropod phylogeny and evolution. Here we compare complete mitochondrial genomes to show that Collembola, a wingless group traditionally considered as basal to all insects, appears instead to constitute a separate evolutionary lineage that branched much earlier than the separation of many crustaceans and insects and independently adapted to life on land. Therefore, the taxon Hexapoda, as commonly defined to include all six-legged arthropods, is not monophyletic.

The phylum Arthropoda comprises the major groups Hexapoda (insects and presumed allies), Myriapoda (e.g., centipedes and millipedes), Chelicerata (e.g., spiders and horseshoe crabs), and Crustacea (e.g., crabs and lobsters). Many studies have attempted to reconstruct the evolutionary relationships among arthropods using various approaches such as paleontology (*1*), comparative morphology (*2*), comparative developmental biology (*3*, *4*), and molecular phylogenetics (*5*, *6*).

It has long been held that hexapods (*7*) constitute a monophyletic taxon (*8*, *9*) and that their closest relatives are to be found in myriapods (*10*). More recently, molecular and developmental studies have rejected this relationship (*3–5*, *11*, *12*) in favor of a closer affinity between Hexapoda and Crustacea (Pancrustacea or Tetraconata). In this context, special attention must be given to the apterygotes (springtails, silverfish, and their allies), the wingless hexapods thought to branch at the base of Hexapoda. The phylogenetic position of these groups is still unclear (*13–16*), casting doubt even on the monophyly of the Hexapoda (*17*).

A potentially powerful technique for resolving deep relationships is to compare whole mitochondrial genomes (*5*, *17*, *18*). Phylogenetic analysis of the only complete mitochondrial sequence available for an apterygotan species (*17*) suggested the possibility that Collembola might not be included within Hexapoda, contrasting with the classic view of a monophyletic taxon that includes all six-legged arthropods. Collembola have been clustered within crustaceans in other molecular and/or combined data sets (*15*, *16*), but the possible paraphyly of Hexapoda has not been given specific attention and the deserved consideration. We have now sequenced the complete mitochondrial genomes of two additional species (*19*) specifically chosen to address this problem: *Tricholepidion gertschi*, representing one of the most basal lineages of the Insecta (Zygentoma), and *Gomphiocephalus hodgsoni*, another collembolan, to test support for the two competing hypotheses of a monophyletic versus paraphyletic Hexapoda.

An initial phylogenetic analysis performed on the 35-taxon data set (*19*) produced the tree shown in Fig. 1. The tree has high support at most nodes, with support decreasing toward deeper relationships. This analysis strongly supports the Pancrustacea hypothesis, with the exception of the position of *Apis* and *Heterodoxus*. *T. gertschi* is basal to all the pterygotan insects, supporting the monophyly of the Insecta. The four crustacean sequences are divided into two well-defined groups (representing Malacostraca and Branchiopoda), but their reciprocal relationships and position relative to the Insecta are not resolved. The Crustacea + Insecta node is well supported, and it excludes the two collembolans, which cluster together as the basal lineage of the Pancrustacea. A second group unites the Chelicerata + Myriapoda [as in (*20*)] but also includes the insects *Apis* and *Heterodoxus*, presumably as an artefact.

Although this tree shows many interesting outcomes, it also contains some evidently untenable relationships, which nevertheless have strong statistical support. This indicates the presence of anomalies in the evolution of these sequences that introduce strong systematic errors in the analysis. The most likely factors that can cause these anomalies are unequal base composition [which can bias amino acid composition (*21*)] and uneven rates of evolution among different lineages. This problem might be especially acute, because some taxa share an extremely high AT bias—*Apis* (84.8%), *Rhipicephalus* (78.0%), and *Heterodoxus* (79.3%)—and different rates of evolution,

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which could potentially cause artefactual attraction (*22*) in this analysis. Such sequences are usually removed from phylogenetic analyses owing to their evidently incorrect placement and disturbance to the reconstruction. To recognize and exclude from the analysis those sequences whose placement in the phylogenetic tree could be influenced by such anomalies in the mechanism of evolution, rather than by the true historical process, we performed a detailed statistical test (*19*) to select a subset of sequences with homogeneous modes of evolution and whose rate of evolution is compatible with that of *Gomphiocephalus* and *Tricholepidion*. The placement of these two taxa is key to assess-

Fig. 1. Maximum-likelihood [ProtML (*24*)] phylogenetic reconstruction, complete data set. Numerals at each node show local bootstrap probability values. Branch lengths are drawn proportionally to maximum-likelihood estimates.


ing the monophyly of the Hexapoda, so it is especially important that the taxa compared are compatible with them. The methods of analysis outlined above, applied to this reduced data set, produced the two trees shown in Fig. 2, which differ only in the placement of *Ostrinia* with respect to the remaining Holometabola. Again, strong support is obtained for the Pancrustacea, with *Tricholepidion* basal to the remaining pterygotan insects, and the two collembolans placed outside the Crustacea + Insecta clade. The trees also show monophyly of Crustacea, although with a lower level of support. *Limulus* is recovered as the sister group of the Pancrustacea, in contrast with the analysis based on the 35-taxon data set, but again with very low support. The resulting trees do not seem to be sensitive to the taxa included (fig. S2).

The most interesting result produced by this study is certainly the nonmonophyly of Hexapoda—that is the position of the two collembolans outside the Crustacea + Insecta clade, agreed upon by all analyses and with high levels of support. To test the relative positioning of Crustacea, Collembola, and Insecta in more detail, we compared two alternative topologies using analytical tests. The hypothesis of Crustacea external to a monophyletic Hexapoda (here, Insecta + Collembola) is strongly rejected (Table 1) in favor of the proposed nonmonophyly of Hexapoda. We also applied the same tests to the problem of the basal trichotomy between Chelicerata, Myriapoda, and Pancrustacea. A sister group relationship between Pancrustacea and Myriapoda (=Mandibulata) is strongly rejected (Table 1), and no significant difference in support was found for the other two possible hypotheses. This accords with the low levels of support found in all trees at this node.

It has been generally accepted that the taxon Hexapoda, including the basal apterygotan orders, is monophyletic. This conclusion is strongly supported by similarities in their body organization (composed of head, thorax, and abdomen), as well as other morphological characters including eye and leg structure and the absence of limbs in one of the cephalic segments (*9*). On the other hand, the interpretation of such characters also depends on which is the closest relative of the Hexapoda, and even on the basal splitting of the latter taxon (*9*). Nevertheless, apterygotan taxa, including Collembola, show a number of peculiar features that at least complicate the analysis of their affinities with the Insecta sensu stricto (*9*, *23*) and leave some room to question these affinities altogether. The acceptance of nonmonophyly of Hexapoda implies that the tripartite and six-legged body plan typical of Hexapoda would be a convergent acquisition of collembolans and the "true insects."
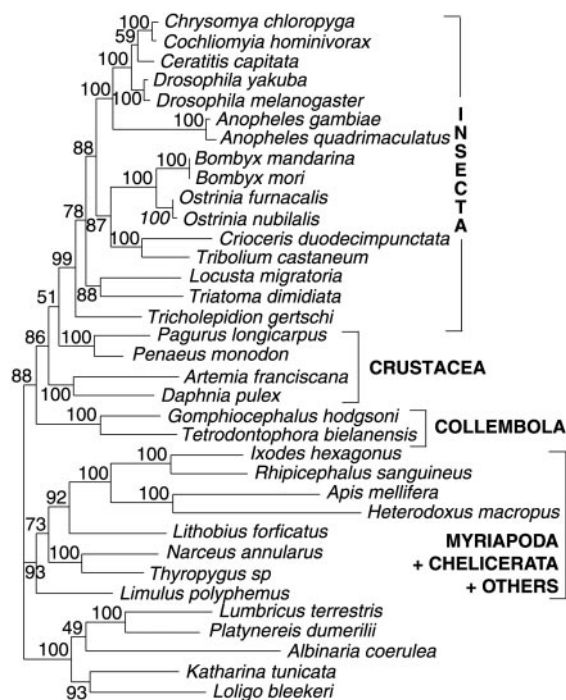
Fig. 2. Maximum-likelihood [ProtML (*24*) and MrBayes (*25*)] phylogenetic reconstructions, reduced data set. Alternative placement of *Ostrinia* follows MrBayes reconstruction. Numerals above each node show local bootstrap probability values (ProtML), and numerals below each node indicate posterior probabilities (MrBayes). Branch lengths are proportionate to maximum-likelihood estimates produced by ProtML.
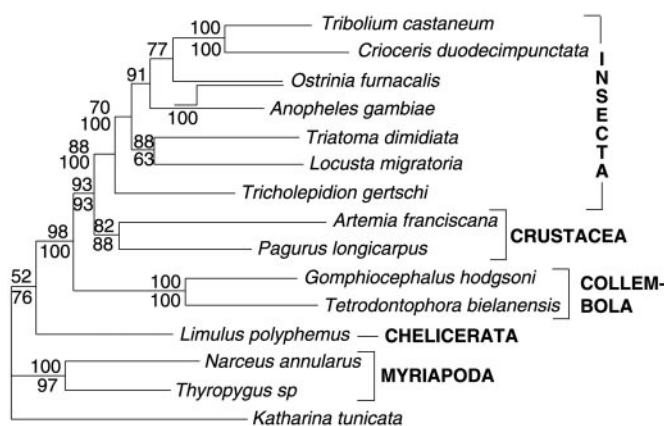

Table 1. Tests of significance for competing hypotheses. Statistical tests of significance were conducted for different competing phylogenetic hypotheses within Pancrustacea and within arthropod classes. au, approximately unbiased test; kh, Kishino-Hasegawa test; sh, Shimodaira-Hasegawa test (*26*).

Tree	−ln L (ProtML)	au	kh	sh	
(Collembola, (Crustacea, Insecta))	19723.73	0.991	0.979	0.979	Best
(Crustacea, (Collembola, Insecta))	19744.96	0.009	0.021	0.021	
(Myriapoda, (Chelicerata, Pancrustacea))	19723.73	0.509	0.496	0.649	Best
((Myriapoda, Chelicerata), Pancrustacea)	19723.97	0.509	0.504	0.626	
(Chelicerata, (Myriapoda, Pancrustacea))	19739.90	0.006	0.032	0.084	

Our analysis, based on a large, specifically targeted data set and modern statistical tools, strongly supports the view that Hexapoda is not monophyletic, that at least some apterygotes have adapted to life on land independently from insects, and that those features shared between some apterygotes and insects might have originated independently in these lineages.

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Role of EphA4 and EphrinB3 in Local Neuronal Circuits That Control Walking

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Local circuits in the spinal cord that generate locomotion are termed central pattern generators (CPGs). These provide coordinated bilateral control over the normal limb alternation that underlies walking. The molecules that organize the mammalian CPG are unknown. Isolated spinal cords from mice lacking either the EphA4 receptor or its ligand ephrinB3 have lost left-right limb alternation and instead exhibit synchrony. We identified EphA4-positive neurons as an excitatory component of the locomotor CPG. Our study shows that dramatic locomotor changes can occur as a consequence of local genetic rewiring and identifies genes required for the development of normal locomotor behavior.

Rhythmic movements such as locomotion and swimming require that muscles contract and relax in a complex repetitive pattern. Central pattern generators, or CPGs, are local spinal neuronal networks that generate and coordinate these rhythmic muscle activities (*1*, *2*). In the fruit fly, it was recently shown that the CPG for peristaltic crawling develops in the complete absence of sensory input (*3*). In two nonmammalian vertebrate species, the lamprey and the *Xenopus* tadpole, the critical neuronal components of the locomotor CPG have been identified (*4*, *5*). In mammals, the CPGs controlling limb movements are located in the ventromedial part of the spinal cord (*6*). However, the neuronal organization is still poorly understood (*2*), and no molecules that contribute to CPG development have been identified. Because CPGs are important for spinal control of walking in humans (*7*), understanding their neuronal organization and molecular determination is essential in the ongoing effort to reestablish locomotor

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Fig. 1. The lumbar segments of the spinal cords of neonatal *ephA4*- and *ephrinB3*-null mice exhibit synchronous left-right ventral root activity. (**A** to **C**) Images of WT mice displaying normal locomotor activity (A) or *ephA4*-null mice (B) and *ephrinB3*-null mice (C) displaying a rabbitlike gait. (**D** to **F**) Recorded activity after application of NMDA and serotonin to the isolated spinal cord (a 4-μM solution of each drug) of WT mice (D), *ephA4*-null mice (E), and *ephrinB3*-null mice (F) in flexor (L2) and extensor ventral (L5) roots. r, right; l, left. (**G** to **I**) Circular phase diagrams derived from 20 locomotor cycles for the WT (G), *ephA4*-null (H), and *ephrinB3*-null (I) mice shown in (A) to (C), respectively, (**J** to **L**) Plots show the vector points of L2 pairings for all experiments conducted on WT mice (*n* = 5) (J, green squares); *ephA4* heterozygotes (*n* = 13) (K, black triangles) and homozygotes (*n* = 14) (K, blue circles); and *ephrinB3* homozygotes (*n* = 9) (L).