The Phylogeny and Developmental Biology of Insects

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ABSTRACT

Arthropod phylogeny has been one of the most contested areas in evolutionary biology. Morphologically, the arthropods appear to be divided into two lineages where the hexapod, myriapods and crustaceans compose one clade, and the chelicerates are basal. However the growing weight of molecular evidence such as mitochondrial gene order and ribosomal DNA sequences have offered an alternative pattern of evolution, placing hexapods and crustacean together, and myriapods and chelicerates in another clade. This study investigates the validity of \textit{Hox} genes as evolutionary markers. Novel \textit{Hox} sequences and some deposited in Genbank from representatives of the main taxa were phylogenetically compared using maximum parsimony, with bootstrap testing of clade stability to determine the usefulness of these genes in resolving these discrepancies. Four undisputed clades were used as guidelines to assess the validities of the various trees produced. Phylogenies of \textit{Hox} genes at three different levels (individual genes, across all genes at nucleotide level and across all genes at the amino acid level) did not produce logical or satisfactorily supported trees. A qualitative examination of individual amino acid changes within the alignment of all genes also offers some evidence against using \textit{Hox} genes as tools to study the evolutionary relationships of arthropods.

INTRODUCTION

There are currently four main extant arthropod taxa: chelicerates, crustaceans, hexapods and myriapods. Phylogenies constructed using morphological characters place the hexapods, crustaceans and chelicerates together, with the myriapods basal to them. Molecular evidence on the other hand supports a hexapod+crustacean and chelicerate+myriapod clade. Mitochondrial and ribosomal gene sequences have both been used as molecular characters for phylogeny work. A recent paper by Cook \textit{et al.} (2001) suggests the use of \textit{Hox} gene cluster to study arthropod evolutionary associations. \textit{Hox} genes are developmental regulatory elements which arose as early as the bilateran split, and have since been conserved in all animals (Cook \textit{et al.}, 2001). In different Arthropods, differential expression of \textit{Hox} genes are linked to the appearance of strikingly different morphological features, thus they may also be interesting in our quest to resolve arthropod relationships (Akam, 2000, Brown \textit{et al}, 2000, Galant and Carroll, 2002). Cook \textit{et al.} (2001)

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analysed the amino acid changes of nine Hox genes in their study, using maximum likelihood. Further analysis to investigate the usefulness of Hox genes can be done by including new information in the form of gene sequences from listed species not included in the original comparison study, as well as novel Hox sequences obtained from new species. This study will compare these sequences at both the amino acid and nucleotide levels, using a maximum parsimony method. This could test (1) the accuracy of Cook et al.’s tree, (2) the usefulness of Hox genes as molecular characters for phylogeny reconstruction and (3) the validity of the 2 clades suggested by molecular evidence.

MATERIALS AND METHODS

Short fragments of the central homeodomain region of Hox genes were amplified using degenerate primers and DNA extracts of eight species of insects from the Ephemeroptera, Plecoptera, Trichoptera and Thysanura families. A reamplification cycle was necessary to obtain good bands. The amplified sequences were then transformed into E. coli for sequencing. Known Hox sequences were also extracted from Genbank and a dataset of nine Hox genes, represented by 19 taxa, was created. Datasets used by Cook et al. were recreated and tested to assess the validity of their reported trees and to evaluate their conclusion that Hox genes are good evolutionary markers. Phylogenetic analysis using MegAlign, MaClade 4.03 and PAUP4.010 was carried out using both nucleotide and amino acid characters with maximum parsimony at 70,000 maxtrees were carried out with bootstrap testing of 500 replicates to determine the degree of support for the trees. Nucleotide characters of the best represented individual Hox genes, AdbB and Dfd, were also tested to determine their usefulness in determining phylogenetic relationships. To evaluate the quality of the data, we tested whether phylogenetic analyses would recover four monophyletic clades that are beyond doubt. These are (1) the Hexapods (2) the winged insects (Pterygota) (3) the Holometabola (Diptera, Trichoptera, Coleoptera and Lepidoptera) and (4) the Chelicerates.

RESULTS

Ten new sequences of six classes of Hox genes from five different species, belonging to the Deformed (Dfd), sex combs reduced (Scr), abdominal (A) (AbdA), abdominal (B) (AbdB), fushi-tarazu (Ftz) and ultrabithorax (Ubx) classes were obtained by amplification using different combinations of six degenerate primers. These were identified as fragments of the homeodomain of Hox genes. The recreation of Cook et al.’s (2001) datasets did not yield trees which matched theirs at all. Nine Hox genes were selected, Abdominal A (AbdA), Abdominal B (AbdB), Antennepedia (Antp), Deformed (Dfd), fushi-tarazu (ftz), labial (lab), proboscipedia (Pb), sex combs reduced (Scr) and ultrabithorax (Ubx). Phylogenetic trees constructed using nucleotide characters of all nine Hox genes represented by 19 taxa, and the two best represented individual genes, AdbB and Dfd, using maximum parsimony generally did not yield consistent or well resolved trees which support the above undisputed groupings. Using amino acid characters to generate a phylogenetic tree also did not yield satisfactorily resolved trees. In all cases, bootstrap testing did not provide good support for
the major clades expected. Qualitative visual examination of the amino acid changes in aligned sequences also does not support the usefulness of Hox genes for phylogenetic analysis.

DISCUSSION


We find that the phylogenetic trees we obtained from maximum parsimony comparison methods do not match at all the trees reported by Cook et al. (2001) at all. Bootstrap analysis also did not provide well supported evidence to support our trees. Therefore, we conclude that the phylogenetic relationships reported by Cook et al. (2001) are not valid, and that their conclusion that Hox genes can be used as good phylogenetic characters is in doubt.

Usefulness of Hox genes as molecular evolutionary characters

A maximum parsimony testing of the nucleotide characters for all nine Hox genes did not yield well supported trees, nor did it help resolve branching discrepancies. Trees constructed from strict consensus were nonsensical based on the undisputed clades mentioned above (Figure 3). For example, a Crustacea and a Chelicerate were placed in the same clade as most of the hexapods and the Pterygota do not appear as a single group, with Tribolium far away from the rest of the Pterygota. Phylogenetic analysis of the two individual Hox genes with the most complete set of taxa with sequences, AbdB and Dfd, also did not yield phylogenetic trees that made sense. Those generated from Dfd (Figure 2(a)) did not reconstruct the expected Holometabola clade, as Bombyx, a Lepidopteran was placed outside the clade, which includes Folsomia, a basal hexapod. The tree generated from AbdB did not support the monophyly of the Hexapods, nor the Holometabola clade. Bootstrap support did not offer support to any of the expected clades. Therefore, the nucleotide characters of Hox genes are not very useful as evolutionary markers.

The phylogenetic tree constructed using the amino acid characters was also not revealing. The Holometabola do not appear together, with Tribolium (Coleoptera) appearing with most of the crustacean, myriapods and chelicerates, and Schisocerca and Bombyx appearing basally. In addition, this tree does not support a monophyletic Chelicerate clade. Qualitatively, a visual comparison of the amino acid alignments of the Hox genes revealed amino acid changes that do not appear to be informative. In three out of four cases, such changes are not useful in resolving phylogeny issues, because the basal Onychophoran appears to have the same amino acid sequences as higher order insects and crustaceans. This could be due to convergent evolution, or because amino acid reversals have taken place in the intervening era since the Hox genes arose.

The persistent lack of resolution of phylogenetic relationships despite the use of different characters and character sets in testing by maximum parsimony and quantitative search methods leads us to conclude that the use of Hox genes as molecular evolutionary markers is not valid. Until more data in the form of more complete Hox sequences for more arthropod species are discovered, Hox genes will not be very useful as subjects of study of the phylogenetic relationship of arthropods.
Validity of the Insecta+Crustacea and Chelicerate+Myriapod clades

Data analyzed at three levels of complexity (individual gene level, nucleotide character and amino acid characters) do not complement each other, and indeed, some times appear to contradict each other. The lack of significant support for most relationships within the various phylogenetic relationships created means that at this point, we are no closer to resolving the phylogeny issue. The validity of the above clades will probably only be conclusively demonstrated in the future, when we are able to integrate all significant morphological, molecular and paleontological characters into a single dataset.

REFERENCES


