

Points of View

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Long-Branch Distraction and the Strepsiptera

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Whiting and Wheeler (1994) proposed that Strepsiptera + Diptera (Halteria) were sister groups based on molecular and morphological data. Since that proposition, there has arisen a controversy as to whether this sister-group relationship can be taken at face value, or whether it is an artifact of long-branch attraction. Although there appears to be no disagreement that these taxa are sister groups in a parsimony analysis, the contention centers around whether we should accept the results of parsimony, or whether we are being "positively misled" by long-branch attraction (Felsenstein, 1987).

The original phylogenetic analysis of Whiting and Wheeler (1994) consisted of 18S rDNA sequenced from 23 exemplar taxa representing all holometabolous orders, with three sequences from the Diptera and four from the Strepsiptera, and an extensive morphological matrix. Whiting and Wheeler found support for a monophyletic Halteria and also found that the remaining ordinal relationships were highly congruent with morphology. These authors concluded that the Halteria appeared to be a well-supported monophyletic group, and discussed the implications these results have on homeotic evolution in Strepsiptera.

Carmean and Crespi (1995) criticized this result by performing a 13-taxon parsimony analysis. Their matrix included one strepsipteran and two dipteran sequences, but lacked representatives of four holo-

metabolous insect orders: Raphidioptera, Megaloptera, Trichoptera, and Lepidoptera. These authors also found a monophyletic Halteria, with long branches leading to Diptera and Strepsiptera that were supported with fairly high bootstrap values. Based on these criteria (long branches and high bootstrap values), they concluded that Strepsiptera + Diptera was an artifact of long-branch attraction.

More recently, Huelsenbeck (1997) reanalyzed the same 13 sequences as Carmean and Crespi (1995) and, through the use of simulation studies and maximum-likelihood analyses, argued that long-branch attraction is a problem with these data. He concluded that when the phylogeny is estimated with a method that is less sensitive to long-branch attraction, the dipteran and strepsipteran branches are separate. Strepsiptera is placed as the sister group to the two beetle sequences, and this result is consistent with the results of morphological studies. Consequently, he argued for using methods that are "less sensitive to the long-branch problem in the first place, especially if application of tests proposed here indicates that long-branch attraction is a prevalent problem in nature."

This reanalysis by Huelsenbeck came (literally) on the tails of the more comprehensive analysis of Whiting et al. (1997). These authors sequenced multiple representatives of all holometabolous insect orders and multiple outgroups for 18S and 28S rDNA, and generated sequences for six

strepsipteran and four dipteran taxa. In addition, they compiled and analyzed the most comprehensive morphological matrix for insect ordinal relationships. This resulted in a matrix of 85 sequences for 18S, 53 sequences for 28S, and 176 morphological characters. Whiting et al. (1997) found that regardless of how the sequences were aligned, or the data were partitioned or combined, Strepsiptera and Diptera always formed a monophyletic group under parsimony analysis. Whiting et al. (1997) further argued that a monophyletic Halteria was not a result of long-branch attraction for two primary reasons: First, there is excellent congruence between the 18S, 28S, and morphological data in support for a monophyletic Halteria, as well as support for the groups Holometabola, Neuroptera, Raphidioptera + Megaloptera, Hymenoptera, Lepidoptera, Trichoptera, and Amphiesmenoptera (Trichoptera + Lepidoptera). Not only is the Halteria consistently recovered in a parsimony analysis of the molecular data, but these other groups are well supported and congruent with the morphology-based phylogenies. Second, the branch lengths for Diptera, Strepsiptera, and Halteria are comparable to those of other insects groups in the analysis when the Holometabola are more densely sampled. For instance, the 18S branch lengths for Diptera (52–82) and Strepsiptera (33–63) are not far out of range of those for Lepidoptera (28–45) and Trichoptera (19–35); the branch lengths for Halteria (11–48) and Amphiesmenoptera (10–32) are even more comparable (Whiting et al., 1997:Fig. 7). For 28S the situation is actually reversed: In Lepidoptera the branch length (23–48) is greater—and with higher Bremer support—than in Diptera (14–27) and Strepsiptera (20–24); likewise the branch length for Amphiesmenoptera (10–37) is greater than for Halteria (9–21). This is significant for two reasons: (1) The long branches of Lepidoptera and Trichoptera are never attracted to those of Strepsiptera and Diptera; each of these groups are always monophyletic, as are Halteria and Amphiesmenoptera. This indicates that these sequences are not just randomly

grouping based on shared elevated substitution rates; there is some signal present that is being recovered by parsimony. (2) No entomologist doubts the monophyly of Amphiesmenoptera: It is supported by well over 20 morphological characters. Hence, no one appears alarmed that there are long branches supporting Amphiesmenoptera because this is entirely congruent with morphology. Nonetheless, under the criterion of Carmean and Crespi (1995), this result should also be discarded as an artifact of long-branch attraction. Whiting et al. (1997) also presented morphological evidence congruent with the hypothesis of halterian monophyly and concluded that the placement of Strepsiptera as sister group to Diptera is the best supported hypothesis given all the current evidence.

The question raised by the analyses of Huelsenbeck (1997) is whether the more comprehensive results of Whiting et al. (1997) can be taken at face value, or whether Whiting et al.'s results can also be attributed to long-branch attraction. I have two serious problems with these analyses: First, they are based on a presupposition of what the correct phylogenetic outcome should be (namely, Strepsiptera should be associated with Coleoptera, or at least not associated with Diptera); and second, the analyses are based on a molecular matrix that is clearly inadequate for reconstructing holometabolous relationships.

Huelsenbeck makes two interesting assumptions in his abstract. First, his analyses are predicated on the assumption that Strepsiptera and Diptera should not go together. This is the model on which the simulations are based. He then uses a congruence measure, to assay the correctness of his analysis. But Huelsenbeck is myopic on two points: (1) He is apparently not aware of the paucity of evidence supporting the Coleoptera + Strepsiptera hypothesis; and (2) he has only considered the position of Strepsiptera and not assessed congruence with the other taxa in his analyses. This second point is crucial in that there are other holometabolous relationships about which there is little or no controversy,

TABLE 1. Summary of the morphological evidence supporting the phylogenetic placement of Strepsiptera among the holometabolous insect orders. Only one character supports placement with Coleoptera; 13 characters support placement within the Mecoptera and Antliophora. Of these 13, 7 are based on the optimization of missing/inapplicable data for Strepsiptera (4–5, 10–14). For details of characters and optimization, see Whiting (1997).

Synapomorphies for Strepsiptera + Coleoptera

1. Posteromotorism.

Synapomorphies for Placement in Mecoptera^a

2. Ovipositor vestigial or completely lost.
3. Outer pterothoracic coxal remotor absent.
4. Larval cranial cardo-promotor, stipitlacinal, and stipitogaleal muscles absent.
5. Cranial antagonist of the primitive craniolacinal muscle in larva present.

Synapomorphies for Placement in Antliophora^b

6. Dagger-like mandible with anterior articulation reduced.
 7. Prelabium without endite lobes/ligula and associated muscles.
 8. Male abdominal segment IX ring-like, enlarged and fused on the pleuron.
 9. Sperm pump present.
 10. Tentorial muscles of prelabium lost.
 11. Transverse muscle between profurcal arms.
 12. Lateral labral retractor absent.
 13. Tentorial adductors of cardo in larva absent.
 14. Retractor of hypopharynx and ventral dilator of salivarium absent.
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^a Trichoptera, Lepidoptera, Siphonaptera, Mecoptera, Diptera.

^b Siphonaptera, Mecoptera, Diptera.

which provide a better indication of phylogenetic congruence than simply looking at the position of the controversial Strepsiptera.

Contrary to Huelsenbeck (1997), the placement of Strepsiptera as sister group to Coleoptera is not consistent with the results of morphological studies. There is only a single character that supports Strepsiptera + Coleoptera: flight by the hind wings (posteromotorism). The excellent morphological work of Kinzelbach (1971, 1990), Kathirithamby (1981, 1991), and Kristensen (1975, 1981, 1991, 1995) has led to the rejection of all other proposed morphological characters for Coleoptera + Strepsiptera. Huelsenbeck cites the coleopterists who favor Strepsiptera + Coleoptera (e.g., Arnett, 1968; also Crowson, 1960, 1980), but not those publications that appraised, reviewed, and refuted their characters and conclusions (Kathirithamby, 1981; Kinzelbach, 1971, 1991; Kristensen, 1995). Likewise, the wing venation characters presented by Kukalová-Peck (1991) and Kukalová-Peck and Lawrence (1993) have been shown to be based on misinterpretation of strepsipteran morphology and poor phylogenetic methodology (Whiting and

Kathirithamby, 1995). Certainly the doubts expressed by Kristensen (1975, 1981, 1991, 1995) on the character evidence supporting Strepsiptera + Coleoptera should make anyone wary of assuming that this is the expected phylogenetic outcome.

There is a series of morphological characters that specifically argue against the placement of Strepsiptera as sister group to Coleoptera (Table 1). Of the 13 characters that specifically support the placement of Strepsiptera within the Mecoptera (Diptera + Siphonaptera + Mecoptera + Lepidoptera + Trichoptera), 7 are based upon the optimization of inapplicable or missing data, but 6 are based upon states that can be specifically scored for Strepsiptera (see Whiting et al., 1997, for scoring and Whiting, 1997, for further character descriptions and alternative optimizations). Parsimony analysis of these and other morphological characters places the Strepsiptera as sister group to (Diptera + (Mecoptera + Siphonaptera)). Hence the desired Coleoptera + Strepsiptera result should be labeled for what it is: congruent with a single character but clearly not with an overall morphological phylogenetic hypothesis.

Moreover, the parsimony and maximum-likelihood trees as generated by Huelsenbeck are largely incongruent with morphology, and incongruent with molecular trees when taxa are more densely sampled. Even when we ignore the placement of Strepsiptera, his maximum likelihood tree (his Fig. 1b) is less congruent with morphology than his parsimony tree (his Fig. 1a). In the parsimony tree, *Polistes* is sister group to Diptera + Strepsiptera, making the Antliophora (Diptera + Mecoptera + Siphonaptera) paraphyletic. Although the placement of the sawfly at the base of the Mecopterida is congruent with morphology, the paraphyletic Hymenoptera is not. In addition to the preceding paraphyletic groupings, the maximum-likelihood tree displays a paraphyletic Coleoptera + Neuroptera. Maximum likelihood gave him the Strepsiptera + Coleoptera relationship, one not supported by morphological data, but it did not recover other well-supported holometabolous relationships as well as parsimony did.

In any event, both the parsimony and maximum-likelihood trees are poor representations of holometabolous phylogeny, and this can be attributed to my second major criticism: meager taxonomic sampling and the lack of key phylogenetic groups. The Megaloptera, Raphidioptera, Trichoptera, and Lepidoptera are absent. The *Aedes* sequence is full of sequencing artifacts and should be excluded (see Whiting et al., 1997). The Strepsiptera is only represented with a single sequence and the Diptera by *Drosophila* and the problematic *Aedes*. With the exception of Hymenoptera, none of the orders are represented by what entomologists consider basal ordinal taxa, and each order is represented by only one or two sequences. The outgroups selected are also not ideal and one can certainly take issue with the rooting of his trees. The absence of Trichoptera and Lepidoptera is particularly disturbing because, as described earlier, they also have long branches and are in a key phylogenetic position as the sister group to the Antliophora (Diptera + Siphonaptera + Mecoptera). The absence of these critical taxa and the

undersampling of each order oversimplify the issue of which groups have long branches and which have short. In reality, there is a spectrum of long to short branches within the Holometabola, depending on which taxa are sampled and which molecule is considered, and it is not simply the problem of Diptera and Strepsiptera being long and the other taxa being very short. Adding more taxa to the 13 analyzed by Huelsenbeck can only reduce the potential of long-branch attraction, not only by shortening the troublesome branches, but by demonstrating that other groups with long branches are also congruent with morphology under parsimony analysis, as is Strepsiptera + Diptera.

Huelsenbeck has grossly oversimplified the issue of holometabolous phylogeny by analyzing a data set that by its meager size and sparse sampling cannot hope to adequately capture relationships among the Holometabola. Moreover, his maximum-likelihood results are largely incongruent with morphology and are actually worse than his parsimony tree using his own criterion of congruence. Thus the cautionary tale he tells about long branches and parsimony appear to me to be a cautionary tale about the use of maximum likelihood. Certainly his simulation and maximum-likelihood studies limit the size of the matrix he can realistically work with, but this is exactly the point: Why should simulations and manipulations of an oversimplified data matrix take precedence over a more thorough phylogenetic survey? With the Amphiesmenoptera as a prime example, we should not be surprised to find cases of sister groups that have high rates of nucleotide substitution; relative rates of substitution are certainly influenced by phylogeny, and shared elevated rates could indeed be evidence of shared history. The supposition that clades that are the best supported by character data are the ones we should be most suspicious of has the strange result of entailing an inverse relationship between phylogenetic evidence and phylogenetic conclusions. If we discard Strepsiptera + Diptera based on his analyses, we should also discard a mono-

phyletic Hymenoptera, Antliophora, and Coleoptera + Neuropterida. I find no evidence from his analysis to refute the monophyly of Halteria.

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REFERENCES

CARMEAN, D., AND B. J. CRESPI. 1995. Do long branches attract flies? *Nature* 373:666.

FELSENSTEIN, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. *Syst. Zool.* 27:401–410.

HUELSENBECK, J. P. 1997. Is the Felsenstein zone a fly trap? *Syst. Biol.* 46:69–74.

KATHIRITHAMBY, J. 1989. Review of the order Strepsiptera. *Syst. Entomol.* 14:41–92.

KATHIRITHAMBY, J. 1991. Strepsiptera. Pages 684–695 in *The insects of Australia: A textbook for students and research workers*, 2nd edition (I. D. Naumann, P. B. Carne, J. F. Lawrence, E. S. Nielsen, J. P. Spradberry, R. W. Taylor, M. J. Whitten, and M. J. Littlejohn, eds.). CSIRO, Melbourne Univ. Press, Melbourne.

KINZELBACH, R. K. 1971. Morphologische Befunde an Fächerflüglern und ihre phylogenetische bedeutung (Insecta: Strepsiptera). *Zoologica* 119(1/2):1–256.

KINZELBACH, R. K. 1990. The systematic position of Strepsiptera (Insecta). *Am. Entomol.* 36:292–303.

KRISTENSEN, N. P. 1975. The phylogeny of hexapod "orders." A critical review of recent accounts. *Z. Zool. Syst. Evolutionsforsch.* 13:1–44.

KRISTENSEN, N. P. 1981. Phylogeny of insect orders. *Annu. Rev. Entomol.* 26:135–157.

KRISTENSEN, N. P. 1991. Phylogeny of extant hexapods. Pages 125–140 in *The insects of Australia: A textbook for students and research workers*, 2nd edition (I. D. Naumann, P. B. Carne, J. F. Lawrence, E. S. Nielsen, J. P. Spradberry, R. W. Taylor, M. J. Whitten, and M. J. Littlejohn, eds.). CSIRO, Melbourne Univ. Press, Melbourne.

KRISTENSEN, N. P. 1995. Forty years' insect phylogenetic systematics. *Zool. Beitr. N. F.* 36:83–124.

KUKALOVÁ-PECK, J. 1991. Fossil history and the evolution of hexapod structures. Pages 141–179 in *The insects of Australia: A textbook for students and research workers*, 2nd edition (I. D. Naumann, P. B. Carne, J. F. Lawrence, E. S. Nielsen, J. P. Spradberry, R. W. Taylor, M. J. Whitten, and M. J. Littlejohn, eds.). CSIRO, Melbourne Univ. Press, Melbourne.

KUKALOVÁ-PECK, J., AND J. F. LAWRENCE. 1993. Evolution of the hind wing in Coleoptera. *Can. Entomol.* 125:181–258.

WHITING, M. F. 1997. Phylogenetic position of Strepsiptera: Review of molecular and morphological evidence. *Int. J. Insect Morphol. Embryol.* (in press).

WHITING, M. F., J. C. CARPENTER, Q. D. WHEELER, AND W. C. WHEELER. 1997. The Strepsiptera problem: Phylogeny of the insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Syst. Biol.* 46:1–68.

WHITING, M. F., AND J. KATHIRITHAMBY. 1995. Strepsiptera do not share hind-wing venational synapomorphies with the Coleoptera. *J. NY Entomol. Soc.* 103:1–14.

WHITING, M. F., AND W. C. WHEELER. 1994. Insect homeotic transformation. *Nature* 368:696.

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Lagomorphs Misplaced by More Characters and Fewer Taxa

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With technical advancements in the collection of molecular data, there are two main ideologies as to how time and money

for data collection should be allocated. One can collect more data from a few representative taxa (e.g., D'Erchia et al., 1996), or, alternatively, limited data representation from a wide range of taxa can be obtained (Chase et al., 1993). Clearly, both approaches have limitations and advantages, and thus most phylogenetic studies seek to

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