DISTANCES, ASSUMPTIONS AND SOCIAL WASPS

James M. Carpenter[1]

[1] *Department of Entomology, American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024, U.S.A.*

The use of distance data in phylogenetic inference retains adherents in molecular systematics, despite the well-established cladistic critique of distances. Molecular distance matrices have been supposed to measure evolutionary divergence; however, trees based upon analyses of such matrices are generally uninterpretable as doing so—under realistic conditions (Farris, 1981, 1985, 1986). In addition, conversion of character data (for example, molecular sequences) to distances discards information (e.g. Farris, 1981). Adherents of distances have usually defended the approach by invoking restrictive assumptions (for example, additivity in expectation, so that the distances may be interpreted as statistical estimates of actual evolutionary divergence; Felsenstein, 1984, 1986). It has also been claimed that conversion of character data to distances may be preferable under certain circumstances (Swofford and Olsen, 1990: 422, 425), because: (1) sequences are not intuitively meaningful; (2) presuppositions about evolutionary processes can be invoked to estimate unseen events (i.e. correct for non-additivity); and (3) numerous methods for distance analyses exist. A superficially more critical "reason" for preferring distances is the argument that parsimony uses only a small fraction of the data, by which is meant the informative characters (e.g. Li and Graur, 1991: 114). Redolent as this argument is of syncretist "criticism" of cladistic classification, it is actually based upon simulations "demonstrating" that parsimony may give incorrect results, relative to distance methods predicated upon the simulated conditions. That is to say, the argument is a conclusion following from the assumptions of the simulations. All of these defenses of distances are thus alike, in relying upon *a priori* assumptions about evolutionary processes. But which assumptions should be made? And suppose that the assumptions are violated—how may this be determined?

Most studies using distance analyses do not consider these questions at all. Examination of the assumptions of a given distance analysis is instructive, for the data themselves frequently provide evidence that the assumptions are violated. The analysis is then internally contradictory, a point which is emphasized when the results are contrasted with the results from a distance analysis that is not predicated on the same assumptions. Such cases do not only support the preference for character analysis over distances, they argue against *a priori* assumptions in phylogenetic inference in general. I will illustrate this with an example from a recent study on social wasps.

Example

Schmitz and Moritz (1990) presented data on mtDNA restriction fragment size polymorphism for six species of European Vespinae: *Vespa crabro, Vespula rufa, V.*

germanica, V. vulgaris, Dolichovespula media and *D. saxonica*. They calculated "genetic distance" among the taxa using the methods of Nei and Li (1979) and Upholt (1977) and clustered on the resulting matrix using UPGMA. From their phenogram (Fig. 1) they concluded that *Vespula* and *Dolichovespula* are monophyletic genera, that *Vespa crabro* and *Dolichovespula* are closely related, and that *Vespula germanica* is more closely related to *V. rufa* than to *V. vulgaris*, that is, that the division of *Vespula* into *rufa* and *vulgaris* (including *germanica*) species-groups is not supported.

Examination of the data (Schmitz and Moritz, 1990: fig. 3) and reanalyses were done using the PHYSYS system by J. S. Farris and M. F. Mickevich, as implemented on a VAX 8530 running VMS 4.7 at Harvard University.

DATA

As noted by Schmitz and Moritz (1990: 1070), the distances which they calculated are non-metric, that is, the triangle inequality is violated. They did not mention how often. There are nine violations of the triangle inequality in the matrix as determined by the MTEST routine in PHYSYS. This is a very large number for so few taxa, for which the number of possible three-way combinations is 20. Schmitz and Moritz (1990: 1071) justified their use of UPGMA clustering by claiming "With this method the principle of triangle inequalities is not required", citing Nei (1987). The claim is absurd. The use of UPGMA requires *ultrametricity*, a more stringent condition than metricity (see Farris, 1981, 1985). That is to say, for UPGMA to infer phylogenetic relationship accurately, the distances which are being clustered must have diverged at a constant rate. *Any* phenetic clustering is predicated upon the premise that mutually most similar taxa are most closely related. The requirement of ultrametricity is a necessary logical consequence. The triangle inequality is simply a way of determining whether a given matrix meets this prerequisite for
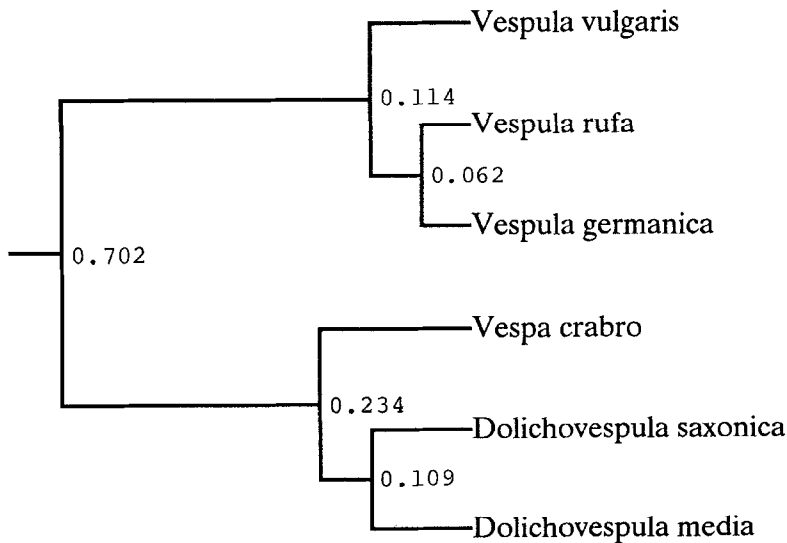

Fig. 1. UPGMA phenogram for the distance matrix of Schmitz and Moritz (1990). Those authors drew their tree so as to indicate the "genetic distances" assigned to each branch; here, the conventional clustering levels are depicted.

phenetic clustering: non-metric matrices cannot be ultrametric. Non-metricity of a distance could be attributed to sampling error (Nei always does so), but this still requires that the distance in question be additive, that is, the distances must sum to the statistical expectation of the true evolutionary distance (Felsenstein, 1984, 1986). Not only can the requirement of additivity not be met in expectation (Farris, 1985, 1986), the many published data sets showing non-metricity provide overwhelming evidence against the *ad hoc* argument of sampling error (Farris, 1985). The very extreme non-metricity of Schmitz and Moritz's matrix likewise vitiates any interpretation of sampling error, and so the data themselves indicate that there is no justification for the use of UPGMA.

REANALYSIS

Distance analyses can be done without the presupposition of rate-constancy that phenetic clustering makes. If results from clustering methods free of the assumption of rate-constancy show clock-like rates of divergence, that is not then an artifact of the method. Conversely, a result of non-clock-like divergence is evidence against the assumption (see Carpenter, 1990, for fuller discussion). Rate-independent clustering, even for non-metric distances, can be done through least-squares fitting. The percent standard deviation statistic (%SD; Fitch and Margoliash, 1967) is that most widely used in assessing fit for distance trees. Schmitz and Moritz did not provide fit statistics for their phenogram, but stated (p. 1071) that "Other parsimonious trees [sic] (Fitch-Margoliash, mixed Wagner-Sokal, PHYLIP 3.2 package, provided by Dr Felsenstein) yielded similar topologies". Their phenogram fits the data quite poorly. The % SD is 69.376 for Fig. 1, as determined by the FIT command of PHYSYS. Farris (1981, 1985) has shown that a modified Distance Wagner algorithm optimizes %SD. I therefore used the PWAGNER Distance Wagner routine in PHYSYS to produce trees of low %SD. I calculated 105 trees that fit the
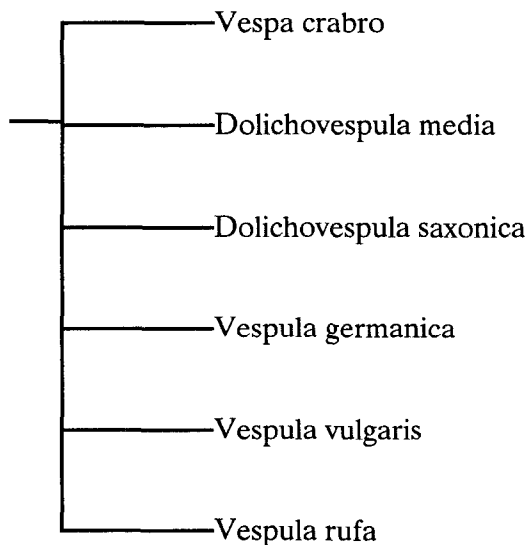
```
          ┌───────Vespa crabro
          │
      ────┼───────Dolichovespula media
          │
          ├───────Dolichovespula saxonica
          │
          ├───────Vespula germanica
          │
          ├───────Vespula vulgaris
          │
          └───────Vespula rufa
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Fig. 2. Strict consensus tree for 105 trees of lowest %SD for the data of Schmitz and Moritz (1990).

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                                      ┌──────Dolichovespula saxonica 0.036
              ┌───────────────────────┤ 0.162
              │                       │      ┌────────Vespula vulgaris 0.329
              │                       └──────┤ 0.512
  ┌───────────┤ -15.574                      │       ┌──Vespula rufa 0.021
  │           │                              └───────┤ -0.247
  │           │                                      └──Vespula germanica 0.041
  │           │
──┤           └──────────────────────────────Dolichovespula media -0.094
  │
  └──────────────────────────────────────────Vespa crabro 0.210
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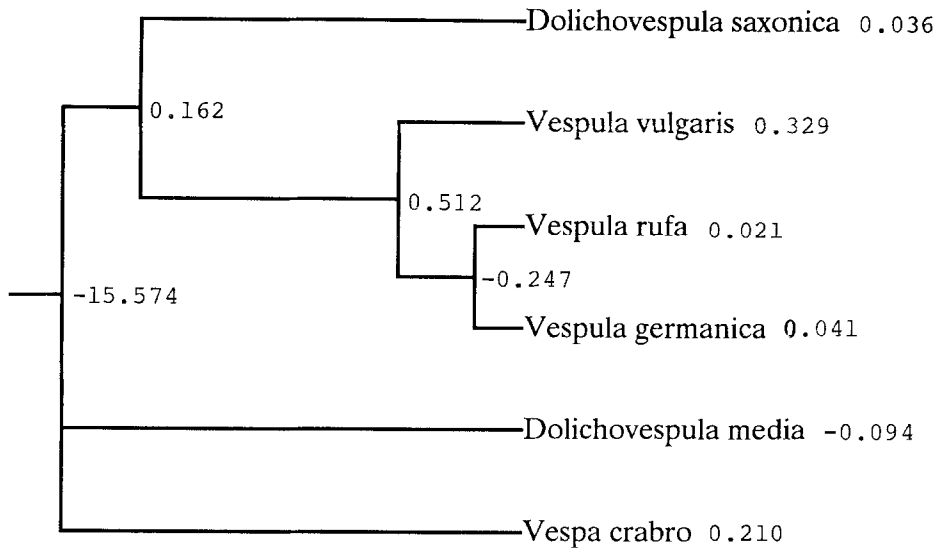
Fig. 3. Best-fitting modified Distance Wagner tree.

data much better (more trees of better fit than Schmitz and Moritz's phenogram undoubtedly exist; 105 is the number obtained when the TRIES command in PHYSYS, which controls PWAGNER, is arbitrarily set to 500); the trees range in %SD from 15.574 to 57.933. The strict consensus of these 105 trees is shown in Fig. 2; it is completely unresolved, indicating substantial ambiguity in the data.

All of the best-fitting trees are alike in having negative branch lengths. Negative branch lengths are methodological artifacts; they are non-sensical. Any distance truly proportional to amount of evolutionary divergence (i.e. additive distances), requires that the branch length assignments on the tree based on the distance be non-negative (Farris, 1981, 1985, 1986). Fig. 3 shows the best-fitting Distance Wagner tree. Note that there are three negative branch lengths, plainly refuting any interpretation of the distances as amounts of evolutionary change. Thus, the distance in Schmitz and Moritz' matrix cannot possibly represent the real genetic divergence between these species (see Farris, 1972, 1981, 1985, 1986, for detailed technical explanation of why this must be so).

JUSTIFICATIONS

Schmitz and Moritz (1990: 1071) acknowledged that their restriction fragment size "method" is not very accurate for investigating intergeneric relationships, but stated that their results "fit nicely into some of the previously published models of wasp phylogeny, though not into others". They cited five "models" (Beaumont, 1958; Yamane, 1976; Greene, 1979; Varvio-Aho et al., 1984; Carpenter, 1987b) in their discussion of three conclusions from their phenogram. I discuss these three points below, further demonstrating that Schmitz and Moritz's conclusions are un-supported.

1. Monophyly of *Vespula* and *Dolichovespula*. Most authors have agreed on this point (review in Carpenter, 1987b). Yamane's (1976) analysis was unable to resolve

monophyly of *Vespula*, but only Varvio-Aho et al. (1974) concluded that these genera were non-monophyletic. Carpenter (1987a) showed that this conclusion was based upon faulty analysis, and Carpenter (1987b: 423–425) listed 19 apomorphic characters supporting the monophyly of the two genera (Schmitz and Moritz, 1990: 1069 incorrectly stated that my cladogram was "based on seventeen morphological characters". I presented several analyses; that is only the first of them discussed.) The monophyly of the two genera may thus be considered as well established, but as Fig. 2 demonstrates, Schmitz and Moritz's data are ambiguous on this issue. Further emphasizing the uninterpretability of these distances, the best-fitting tree (Fig. 3) does not accord with monophyly of *Dolichovespula*, despite the abundant evidence that this genus is in fact monophyletic.

2. Close relationship between *Vespa* and *Dolichovespula*. Beaumont (1958), Yamane (1976) and Carpenter (1987b) all concluded that *Dolichovespula* is more closely related to *Vespula* than *Vespa*. Carpenter (1987b: 423) adduced four synapomorphies supporting a sister-group relationship between *Dolichovespula* and *Vespula*, and four more that clustered these two genera with *Provespa* to the exclusion of *Vespa*. In support of their conclusion, Schmitz and Moritz cited Green [sic] (1979), but as Carpenter (1987b: 414) pointed out, Greene's paper simply confused character polarity with phylogenetic relationship, and in any event, would have based close relationship between *Vespa* and *Dolichovespula* on symplesiomorphy.

3. Division of *Vespula* into the *rufa* and *vulgaris* species-groups. Monophyly of these two species-groups is an inveterate view, dating back to Bequaert (1930). Carpenter (1987b: 425) cited 12 apomorphic characters supporting this conclusion, in addition to six more synapomorphies establishing sister-group relationships of these two species-groups to the *squamosa* and *koreensis* species-groups, respectively. Schmitz and Moritz, however, concluded that *Vespula germanica*, a member of the *vulgaris* species-group, is more closely related to *V. rufa* than to *V. vulgaris*. Again (Fig. 2), Schmitz and Moritz's data are actually uninformative on this issue. And although the best fitting tree (Fig. 3) seemingly accords with their conclusion, the branch subtending *V. germanica* and *V. rufa* has a negative, hence artifactual, length.

Conclusion

Schmitz and Moritz (1990: 1069) begin their paper with the salutation "MtDNA is a powerful tool for reconstructing the phylogenetic relationships between and within species". Their analysis certainly failed to demonstrate this. The assumption of their procedure is contraindicated by their own data, and their results do not in fact accord with other phylogenetic evidence. Reanalysis without the assumption shows the distance data to be relatively uninformative on phylogenetic relationships, and thus of little value. This result is the same as for reanalyses of other distance data sets for social wasps (Carpenter, 1987a, 1990). It adds yet another datum for the conclusion that distances are generally poor phylogenetic indicators.

Acknowledgements

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