Of Genetic Distances and Social Wasps

James M. Carpenter


Stable URL:
http://links.jstor.org/sici?sici=0039-7989%28199012%2939%3A4%3C391%3AOGDASW%3E2.0.CO%3B2-7

Systematic Zoology is currently published by Society of Systematic Biologists.

Your use of the JSTOR archive indicates your acceptance of JSTOR’s Terms and Conditions of Use, available at http://www.jstor.org/about/terms.html. JSTOR’s Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at http://www.jstor.org/journals/ssbiol.html.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is an independent not-for-profit organization dedicated to creating and preserving a digital archive of scholarly journals. For more information regarding JSTOR, please contact support@jstor.org.
Points of View


Of Genetic Distances and Social Wasps

JAMES M. CARPENTER

Museum of Comparative Zoology, Harvard University,
Cambridge, Massachusetts 02138

The use of genetic distances as phylogenetic indicators is declining among systematists. In part this is due to the critique of distance data offered by Farris (1981, 1985, 1986), who showed that distances are generally uninterpretable as amount of evolutionary change for a variety of reasons. Chief among these are lack of metricity and lack of additivity under realistic circumstances, facts that obviate the most common method of analysis of distances, namely phenetic clustering on Nei’s (1972) genetic distance. The use of genetic distances is undoubtedly also declining due to recent advances in methods for direct analysis of nucleotides. Technology for sequencing and restriction site mapping is now readily accessible, and provides a potentially more powerful source of data for phylogeny reconstruction than distances (see Hillis and Moritz, 1990). Moreover, nucleotide data can be analyzed with a theoretically defensible technique, parsimony.

The foregoing is not to say that genetic distances are without any value in phylogenetic reconstruction. There is a correlation between such distances and phylogeny, but that correlation is far from perfect. The branch lengths of phylogenetic trees constructed from distances may not be interpretable as amount of evolutionary divergence, but the topological relationships depicted by the trees may perhaps be accurate. The analysis of such data should therefore be performed with care, and clustering methods should not be predicated on unsupportable premises. In particular, clustering methods should not make presuppositions about rates of evolutionary change, a point now widely accepted by molecular systematists (e.g., Berlocher, 1984; Buth, 1984; Hillis, 1987; Hillis and Moritz, 1990). Unfortunately, this point is as yet little appreciated among population geneticists. In population genetic studies, phylogenetic inferences are still commonly made from phenetic clustering on genetic distance data, as scanning current issues of a journal such as Evolution shows. The use of this procedure may reflect the fact that phylogenetic reconstruction is not the primary object of population genetics, and so little attention is paid to systematic methods. However, even if done as an afterthought, phylogenetic inferences should be made using proper techniques.

In this paper, I will outline the general argument against the use of phenetic clustering on genetic distances, illustrating the argument with reference to a recent example employing these techniques in social wasps. I will then present a reanalysis of the example data, using techniques that are suggested to be more appropriate. The reanalysis will show that the genetic distances convey very little phylogenetic information.

THE EXAMPLE

The example is a study by Nozawa and Itô (1989). These authors presented data on electromorph frequency for 25 loci
screened in nine species and 12 populations of Japanese paper wasps: *Ropalidia fasciata*, *Parapolybia indica*, *Polistes* (*Mega-
ropolistes*) *jadwiga*, *P. (M.) rothneyi*, *P. (Polistella) snelleni*, *P. (P.) mandarinus*, *P. (P.) japonicus*, *P. (Polistes) chinensis*, and *P. (P.) riparius*. Asserting that the assumption of a molecular clock was applicable to these data, Nozawa and Itô calculated Nei’s (1975) distance between each pair of taxa, and clustered on the resulting matrix using UPGMA and WPGMA. They concluded that they had identified four phylogenetic lines (viz. *Ropalidia*, *Parapolybia*, the nominate subgenus of *Polistes*, and *Mega-
polistes* + *Polistella*), the interrelationships of which could not be resolved due to differing placements of *Ropalidia* on each of their phenograms (Figs. 1, 2). Nozawa and Itô (1989) further concluded that the nominate subgenus had “diverged remarkably from the other two subgenera” (p. 193) and characterized this as a “large genetic differentiation, large enough to be almost inter-
generic” (p. 194). Although not explicitly mentioned by Nozawa and Itô, the implication of their phenograms is that the ge-
nus *Polistes* itself is not monophyletic. Fi-
nally, they also concluded that *Polistella* is paraphyletic in terms of *Mega-
polistes*.

Nei’s distance had previously been em-
ployed in phylogenetic inference in social wasps only by Varvio-Aho et al. (1984), in a study of European Vespinae. That study was criticized by Carpenter (1987) on a number of grounds. I demonstrated that the conclusions of Varvio-Aho et al. rested upon faulty analysis, and that when ana-
alyzed correctly their data were relatively uninformative on phylogenetic rela-
tionships. Nozawa and Itô (1989) cited this pa-
er, but stated that my criticism “seems to be due to his rejection of this molecular clock assumption” (p. 184). This is mis-
leading; most of my criticism had to do with metricity and additivity. The same criticisms are applicable to the paper by Nozawa and Itô, and it will be seen that their data are also uninformative on rela-
tionships. Character analyses resolving the relationships among *Polistes*, *Ropalidia*, and *Parapolybia* are presented elsewhere (Car-
penter, 1990, and in prep.).

THE CASE AGAINST PHENETIC CLUSTERING

Phenet methods, which proceed by clustering together mutually most similar taxa, were recommended for analyzing gen-
etic distance by Nei (1972), under a model based on the presupposition of a molecular clock for electromorph data. Given con-
stant rates of change, Nei’s distance is propor-
tional to relative propinquity of de-
scent. Phenetic clustering would then ac-
curately infer phylogenetic relationship, as taxa separated by small distances (mu-
tually most similar) would indeed be close-
ly phylogenetically related. However, giv-
en any deviation from clocklike rates of divergence, phenetic clustering is the wrong method, for most similar taxa are then not necessarily most closely related. The case against the use of phenetic clus-
tering, and for the use of methods that do not incorporate the presumption of rate constancy of molecular evolution, is estab-
lished on at least three grounds.

1. Assumption of a Molecular Clock

First, the assumption of a clock merits criticism. The justification for the notion of constant rates of divergence arose out of the neutral theory of molecular evolution, but whether that theory is correct or not, the evidence against completely ho-
mogeneous change is overwhelming (e.g., Thorpe, 1982; Britten, 1986; Hillis, 1987; Wheeler and Honeycutt, 1988; and cf. Lew-
in, 1990). Britten (1986) demonstrated that rates of silent substitutions in DNA may differ by a factor of five or more among clades, and Wheeler and Honeycutt (1988) documented extensive cosubstitution maintaining secondary structure in rRNA. Fitch’s covariation principle of amino acid replacement establishes that evolution of proteins will likewise show rate differences and nonrandom change. This is doc-
umented in the review by Thorpe (1982). Contemporary proponents of a molecular
clock, such as Thorpe, characterize it as at best stochastic since it is dependent upon the idea that the probability of an amino acid substitution is at least approximately constant and thus the accumulated number of substitutions is roughly proportional to time. It cannot therefore be regarded as a true clock with substitutions taking place at regular time intervals [Thorpe, 1982:140]. Thorpe (1982) pointed out that the rate of change for each protein must be calibrated separately before the protein can be used as a clock (p. 142), and so his concept of a clock reduces to no more than that there is a “general relation between taxonomic divergence and evolutionary time” (p. 150). The implication for phylogenetic methods is clear. Stochastic deviations from constant rates of divergence will occur under such a “clock,” hence most similar taxa may not be most closely related, and clustering methods free of the presumption of rate constancy should therefore be employed. Nozawa and Itô (1989) provided no evidence for constant rate of evolutionary change for the enzymes they studied. Calibration for a clock based on Nei’s distance is in any event not empirically feasible at present (Hillis and Moritz, 1990), a situation leading Hillis and Moritz to conclude (1990:512) that “estimates of time based on Nei’s D are probably no better than arbitrary guesses.”
2. Metricity and Additivity of Nei's Distance

The controversial nature of the "clock" is in fact a separate issue from the suitability of phenetic clustering on Nei's distance. In order for a distance measure to be proportional to evolutionary time, it must satisfy the triangle inequality: the distance between any pair of taxa cannot be greater than the summed distances between them and a third taxon. This is the condition of metricity. For a distance measure to be clocklike, it must be ultrametric: the distance between any pair of taxa cannot be greater than either distance between one of them and a third taxon. Nonmetric distances consequently cannot be clocklike; the latter condition is more restrictive. Nonmetric distances may be identified by the assignment of negative branch lengths in best-fitting trees calculated from the distances. Negative branch lengths are methodological artifacts in that they are nonsensical: distances proportional to amount of evolutionary divergence must be additive, hence the branch length assignments must be nonnegative. Discussion of all of these points may be found in Farris (1981, 1985, 1986). As he observed, many published matrices of Nei's distances show violations of the triangle inequality. This is also true of the data of Nozawa and Itô (1989); 12 triplets in their matrix violate the triangle inequality. Nei et al. (1983) argued that nonmetricity of Nei's distance is due to sampling error, assuming that Nei's model is true. But, as Farris (1985) observed, nonmetricity could also obtain if Nei's model is false (Felsenstein [1984] made a similar point)—and the model itself is quite unrealistic (see, for example, Hillis, 1984). The great abundance of data sets showing nonmetricity for Nei's distance renders the explanation of sampling error untenable, and as I observed previously (Carpenter, 1987) this measure should be abandoned. But, if this distance continues to be used, it should be analyzed properly. Whatever the source of nonmetricity, its existence clearly obviates the use of clustering methods that operate under the presumption of strict rate constancy. Unless methods free of this assumption are employed, investigators can never know if they are not being misled by "sampling error."

3. Testing

There is, finally, the basic scientific requirement that assumptions be open to testing as an argument against the use of methods assuming rate constancy with Nei's distance. Whether the molecular clock is true or not, it is an empirical proposition. As such, it can never be tested unless evidence of rate heterogeneity, if that exists, is permitted to be discovered. Phenetic methods cannot permit such a discovery, for they always cluster as if rates of change were constant. By contrast, clustering methods free of the assumption of rate constancy could provide empirical support for a clock; if they show clocklike rates of divergence, that is not an artifact of the method. Similarly, Nei's model is (or ought to be) an empirical proposition. Nonmetricity of Nei's distance may be due to experimental error, but it may be evidence that the model is false. That evidence cannot be discovered using phenetic clustering on Nei's distance, again because of the methodological presumption of rate constancy. Likewise, cases where the model is applicable could potentially be revealed by the use of clustering methods free of this presumption, because the fit of the model is then not an artifact of the clustering method. Obvious as these points may appear to be, they were generally ignored prior to the work of Farris (1981, 1985, 1986). Now, however, awareness of these issues is widespread. For example, Hillis (1987) recommended the use of rate-independent clustering methods for molecular data, and observed that this is a potential test of the molecular clock. Proponents of electromorph data such as Buth (1984) advocate the use of rate-independent methods instead of phenetic techniques, while Berlocher (1984) advocated them as a check. Felsenstein (1984, 1986), while arguing for the interpretation of distance data as only statistical expectations
of the amounts of evolutionary change (a view refuted in detail by Farris [1985, 1986]), suggested use of techniques other than UPGMA in order to optimize least-squares fit. Even Nei himself (Saitou and Nei, 1987) now advocates the use of a rate-independent technique for clustering on sequence data. Nozawa and Itô (1989) failed to consider any of these methodological issues. It is clear, however, that whatever one's views on the existence of a molecular clock or the suitability of Nei's distance in phylogenetic inference, they cannot be accorded immunity from testing. Use of phenetic techniques on such distances conflates presupposition and empirical support.

ANALYSIS WITHOUT THE ASSUMPTION OF RATE CONSTANCY

Given that phenetic clustering should be rejected as an analytical technique for Nei's distance, the question arises as to which method should be used. Choice of methods by their relative performance under simulations (e.g., Nei et al., 1983; Saitou and Nei, 1987) is obviously inappropriate for many of the same reasons as discussed above: the results are model dependent. The alternative criterion is goodness of fit. The choice of fit statistic is a difficult problem (see Farris, 1981, 1985), but in distance analyses a least-squares statistic is most commonly used, typically percent standard deviation (Fitch and Margoliash, 1967). This statistic is therefore employed here, denoted as %SD. The statistic is optimized when trees are calculated under a distance Wagner procedure (Farris, 1972), with branch lengths fitted as in Farris (1981) and multiple trees generated (Farris, 1985). Inter alia, the use of this procedure provides further evidence against both the use of phenetic techniques and the interpretability of Nei's distance. Optimal fit cannot generally be attained by phenetic clustering, and attaining it with nonmetric data requires negative branch lengths, which cannot be interpreted as proportional to evolutionary change.

The distance matrix in Table VI (upper half) of Nozawa and Itô (1989) was analyzed using the PHYSYS system of J. S. Farris and M. F. Mickevich, as implemented on a VAX 8530 running VMS 4.5 at Harvard University. The PWAGNER routine was used to produce trees of low %SD. Figures 1 and 2 show, respectively, the phenograms based upon WPGMA and UPGMA presented by Nozawa and Itô.

RESULTS AND DISCUSSION

Nozawa and Itô (1989) did not provide fit statistics for their phenograms; the %SD for the WPGMA tree is 18.308, and that for the UPGMA tree is 16.580. Calculation of 500 trees with the PWAGNER routine resulted in a range of %SD from 9.816 to 13.636. (More trees of better fit than Nozawa and Itô's phenograms undoubtedly exist; this arbitrarily large number is sufficient to illustrate the argument on clustering methods.) These trees are alike in having negative branch lengths. Advocates of distance analyses such as Prager and Wilson (1978) recommend presenting the information common to multiple trees, that is, a form of consensus tree, and Post and Uzzell (1981) suggested the use of a gap in goodness of fit for choosing among trees. The largest gap in goodness of fit for the 500 trees is just 0.237, rather less than the gap between them and the phenograms. The strict consensus for these 500 trees is shown as Figure 3, and it is immediately clear that none of the conclusions of Nozawa and Itô is supported. There are only two resolved groups, and the species Polistes rothneyi is not even one of them. None of the subgenera of Polistes is a group, and the claim of paraphyly of the subgenus Polistella is not supported—that group is merely undefined by these data. Thus, the distance data of Nozawa and Itô are uninformative on relationships of these polistine species. Such uninformative similarity was also the case for the vespine distance data of Varvio-Aho et al. (1984; see Carpenter, 1987). But at least these latter authors also presented their data as characters: the presence/absence of electromorphs. Albeit still ambiguous on generic relationships, the presence/absence coding was rather more informative than the
P. (Polistella) japonicus-A
P. (Polistella) japonicus-SW
P. (Polistella) snelleni-N
P. (Polistella) snelleni-H
P. (Polistes) riparius
P. (Polistes) chinensis
P. (Polistella) mandarinus
P. (Megaplistes) rothneyi-N
P. (Megaplistes) rothneyi-A
P. (Megaplistes) jadwigae
Ropalidia fasciata
Parapolybia indica

FIG. 3. Strict consensus tree for 500 trees of lowest
%SD for the data of Nozawa and Itô (1989).

distance data on specific relationships (cf. Figs. 3 and 5 in Carpenter, 1987). This is
generally the case; the conversion of characters to distances results in loss of information (Farris, 1981; Hillis, 1987). Nozawa
and Itô failed to consider such a possibility, with the result that they simply discarded
data by considering only the much coarser

distances.

One other aspect of the results deserves discussion. That is that, although the
distances are unable to resolve the monophyly of the genus Polistes and its subgenera,
available character data do. Nozawa and Itô (1989) cited Richards (1973) in support of
their contention of paraphyly of Polistella in terms of Megaplistes, to the effect
that Polistella was largely defined by negative
characters, but attempted no analysis. It is worth pointing out that “negative characters” are in fact phylogenetically in-
formative when they represent secondary loss. That is the case for several of the
characters distinguishing Polistella from Megaplistes, as has already been pointed out by
Kojima and Kojima (1988). These characters
include absence of the pronautal fovea and dorsal groove in Polistella, which are
derived conditions within Polistineae (Carpenter, 1989, 1990). Polistella shares these
apomorphies with the subgenus Stenopolistes, and there is reason to infer paraphyly
of Polistella in terms of that subgenus (Kojima and Kojima, 1988), but not in terms of
Megaplistes. The genus Polistes itself is es-

tablished as a monophyletic group by the
autapomorphy of the conical shape of the
first metasomal segment, as shown by out-
group comparison with the Vespinae, sister-
group of the Polistineae (Carpenter, 1981), as well as other genera of Polistineae
(Carpenter, 1990, and in prep.). Additional
apomorphies, convergently derived in
other genera, include the dorsally acute
propodeal orifice and convex larval maxilla. Megaplistes has the apomorphy of the
sacciform digitus of the male genitalia; it
shares this with the subgenus Gyrostoma,
in terms of which it is evidently paraphyle-
etic. Finally, the subgenus Polistes has a
derived reticulate pleural sculpture and a
ventrally evanescent occipital carina, apomorphies it shares with Sulcoplistes, in-
dicating that the social parasites should be
included in the nominate subgenus.

CONCLUSION

Although the particular example de-
tailed here treats social wasps, the points
made concerning methods should be of
general systematic interest. Although sys-
tematists may find these points readily ac-
tceptable, even obvious, clarification of the
methodological arguments may help in
communication with workers in such fields as population genetics. Phylogenetic re-
construction must be based on sound prin-
ciples, and awareness of these principles
must become widespread in other fields for
the importance of knowledge of phylo-
geny to be fully realized in evolutionary
biology.

ACKNOWLEDGMENTS

I thank J. S. Farris, J. Kojima, K. G. Ross, and J. W.
Wenzel for comments on the manuscript. This work
was supported by NSF grants BSR-8508055 and BSR-
8817608.

REFERENCES

BRIFFEN, R. J. 1986. Rates of DNA sequence evolu-
tion differ between taxonomic groups. Science, 231:
1393–1398.
BUTH, D. G. 1984. The application of electrophoretic
data in systematic studies. Annu. Rev. Ecol. Syst.,
Farris on Haeckel, History, and Hull

DAVID L. HULL

Department of Philosophy, Northwestern University,
Evanston, Illinois 60208

I appreciate the efforts of the many people who have written me during the past two years pointing out errors in my Science as a Process, and most were simply that—errors. No differences of opinion were involved, no misunderstandings or subtle meaning changes. Farris (1990) pointed out another. In my book I said that Nelson and