Partial Warps, Phylogeny, and Ontogeny: A Comment on Fink and Zelditch (1995)

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Fink and Zelditch (1995) recently proposed a method by which one may infer phylogeny from ontogenetic transformations of continuous morphological data. They coded ontogenetic regressions of shape on size to obtain discrete characters, which they used as input into a parsimony analysis. As shape variables, they used partial warp scores obtained from an analysis based on thin-plate splines (TPS), which, they had previously argued, could be considered homologous characters and could be used to determine synapomorphies on a phylogenetic tree (Zelditch et al., 1995). Here we discuss several aspects of their protocol that call into question their assertion that the ontogeny of shape (as represented by discrete characterizations of regressions of partial warp scores) is a suitable character set for phylogeny estimation. A similar note (Rohlf, 1998) discusses more theoretical objections to their methodology, and challenges the notion that partial warp scores viewed separately are biologically homologous and interpretable characters. Additionally, we will address two issues raised by Fink and Zelditch (1995). First, does the choice of a different starting form (reference) result in a different phylogeny? Second, does the choice of an alternative basis for tangent space result in a different phylogeny?

The protocol of Fink and Zelditch (1995) is as follows. First, a reference configuration is generated as the mean of several juvenile specimens from a chosen outgroup species. Fink and Zelditch chose the average of three juvenile specimens from one of their outgroups, *Pygopristis denticulata*, as their reference specimen (hereafter called the reference outgroup). They

aligned the specimens using shape coordinates (Bookstein, 1986), choosing the long axis of the fish as the baseline, and calculated the size of each specimen as the square root of the summed squared distances from each landmark to the centroid of that specimen (Gower, 1971). In the absence of allometry, this measure of size, called ''root centroid size'' (Bookstein, 1991), is the only size variable that is uncorrelated with shape (Bookstein et al., 1985; Bookstein, 1991). The difference in shape from the reference to each aligned specimen was then quantified using the TPS (Bookstein, 1989, 1991). TPS allows one to mathematically represent the shape variation present in the configurations of a set of homologous landmarks as variation in a set of parameters of an interpolating function (Bookstein, 1989, 1991), which represents the transformation of the reference to each specimen (Rohlf, 1993). From the reference configuration they calculated the principal warps, which are used to define a set of coordinate axes for tangent space, a linear space approximating the curved shape space, in which the shapes of specimens can be compared using standard linear statistical methods (see Bookstein, 1991; Rohlf, 1996). By projecting the x- and y-coordinates of the aligned specimens onto the principal warp axes, Fink and Zelditch generated a set of shape variables called partial warp scores for each specimen. These variables, as well as two variables representing uniform shape variation, were then individually regressed onto log(centroid size) to calculate ontogenetic shape changes for each species. Fink and Zelditch argued that because the principal warp axes represent shape

change at all noninfinite spatial scales, regressions of shape on size described ontogenetic shape changes at all spatial scales as well.

To obtain discrete characters for their taxa, they coded each ontogenetic shape regression as either a 0, 1, or 2. This was done by determining whether the regression of each partial warp on size was statistically significant, and in what direction (i.e., positive/negative) this shape change occurred. Species with similar changes were assigned similar coding, with the reference outgroup species, *Pygopristis denticulata,* always given the character state 0. For example, two species (*P. denticulata* and *Serrasalmus gouldingi*) both displayed significant positive ontogenetic change for character 20 (partial warp 9y). They each received a 0 for this character, because *P. denticulata* was used as the reference outgroup. The remaining three species (*Pygocentrus cariba, P. nattereri,* and *P. piraya*) displayed no significant shape change for partial warp 9y and were thus coded as 1. Fink and Zelditch then used these coded characters, together with several meristic, myological and osteological characters, in a parsimony analysis of the piranha genus. Finally, they assessed the phylogenetic independence of these characters by identifying the patterns of character change on the cladogram. This was done by examining characters that changed more than once on the cladogram and determining if other characters that changed multiple times diagnosed the same nodes of the phylogeny. If two characters changed multiple times at the same nodes of the phylogeny, they were considered not phylogenetically independent of one another.

The protocol of Fink and Zelditch (1995) allows comparisons of shape in a manner that seemingly combines the concepts of ontogeny and phylogeny. In Figure 2 of their paper, Fink and Zelditch displayed what they define as "net ontogenetic shape changes" for each of their five taxa. These figures were generated by transforming the juvenile form of each species to the adult form of the same species using the thin-plate spline (Zelditch, pers. comm.).

Although we certainly have no difficulties accepting this as an adequate representation of ontogeny, their protocol does not describe ontogeny in the same manner as is depicted in their Figure 2. Their protocol explicitly uses the mean juvenile of one outgroup species as the reference form, and thus the partial warp scores obtained describe the shape changes from a mean juvenile of the reference outgroup species to adults of each of the ingroup species. Because the transformations they are interested in are changes in ontogeny, the primitive (outgroup) condition should not be thought of as a starting form (Fink and Zelditch, 1995:347), but rather as a trajectory in shape space. Therefore, characterizations of ontogeny by partial warps require only the use of a single reference configuration (Zelditch et al., 1995), but not the use of a juvenile configuration.

In a recent study, Naylor (1996) evaluated whether the correct tree topology could be obtained using partial warp scores as data in a phylogenetic analysis. He generated a set of differently shaped imaginary fish that were related by a known, predetermined phylogeny. At each node of the phylogeny, a single unique net shape change was generated, insuring no homoplasy. Naylor calculated partial warp scores for each fish, coded them as multistate characters, and used them to estimate the phylogeny. He found two most parsimonious trees, one of which corresponded to the true tree topology. However, the retention index (Farris, 1989) for these topologies was 0.48, indicating a large degree of homoplasy (eight taxa, 70 characters). When shape characters were mapped onto the phylogeny, Naylor found little correspondence between the partial warp scores and the true shape changes that characterized the taxa. He argued that because each partial warp score represents one geometric aspect of overall shape difference (i.e., a decomposition), the biological realization of such shape differences may not correspond to the individual partial warps, but to a combination of partial warps. When taken in sum, the partial warp scores perfectly describe all shape differences be-

tween the various taxa, but when analyzed individually, this relationship is lost.

It is tempting to assign biological meaning to partial warp axes. However, these axes are simply a convenient mathematical (i.e., geometrically orthogonal) description of tangent space; there is no reason to think they should individually have any specific biological relevance (Naylor, 1996). Biological relevance only comes from the axes taken together, or more accurately, from the morphological space that they span. This was clearly demonstrated by Rohlf (1998), when he showed that a simple shape change between two specimens (an expansion of a subset of landmarks) was not described by any individual warp, but was perfectly described when all partial warps were taken together. Thus the claim by Fink and Zelditch (1995) that partial warp scores should be individually biologically interpretable is unwarranted.

CHOOSING A REFERENCE FORM

In discussing evolutionary transformations, Fink and Zelditch (1995) stated that shape deformations can be interpreted as actual evolutionary events only if an outgroup specimen is used as the starting form. In their protocol, the reference outgroup species served two purposes. First, the average of several juvenile specimens from this species was used as the starting (reference) form for the TPS analysis. Second, the character states of the reference outgroup species were used to code the characters of the other taxa, by coding all regressions of shape on size for the reference outgroup species as 0, and coding those of the ingroup taxa (as well as other outroup taxa) relative to these scores. Such a use of outgroups is common in phylogenetic studies (for discussion, see Maddison et al., 1984; Swofford et al., 1996). Although choosing an outgroup as a reference specimen sounds appealing from an evolutionary perspective, there are a number of difficulties with such a choice. First, the term ''reference configuration'' in geometric morphometrics is somewhat misleading, as it refers to the point of tangency between shape space and tangent space; it should not be thought of as a reference form chosen on biological grounds. The principal warp axes, on which the partial warp scores are based, are defined solely on the basis of the reference configuration, and are very sensitive to different choices of a reference (see Rohlf, 1996, 1998). Because the partial warp scores are merely projections of the specimens onto the principal warps, use of a different reference will yield different partial warp scores for the ingroup taxa. Because Fink and Zelditch did not specify a method by which to choose an appropriate outgroup to serve as the reference (if more than one is available), this poses some difficulties. To illustrate this, we used Fink and Zelditch's protocol to generate four phylogenetic estimates for several host races of the leaf beetle *Neoclamisus bebbianae* (the *Pygocentrus* data were not made available to us). These host-associated races are known to differ both morphologically (Adams and Funk, 1997) and genetically (Funk, 1996), and can thus be considered separate operational taxonomic units for the purposes of this example. The data used here are part of a larger study on the relationship between morphological shape and host-plant specificity (Adams and Funk, 1997). Eleven landmarks were recorded on 30 specimens of *N. bebbianae* from each of four host-plant populations. For each analysis, we chose one of four closely related species (*N. chamaedaphnes, N. comptoniae, N. gibbosus,* and *N. platani*) to serve as the reference outgroup (Funk and Adams, unpubl.). As in Fink and Zelditch, three small individuals from the reference outgroup were averaged to obtain the starting (reference) shape; the remaining 27 specimens of the reference outgroup were used to define shape ontogenies for that taxon. Sixteen partial warp scores were generated from a TPS analysis, and each was regressed on log(centroid size) to determine shape ontogenies for each taxon. These were coded as discrete characters, which were used to generate phylogenetic estimates using a heuristic search and Wagner parsimony, with 100 bootstrap replicates, in PAUP 3.1 (Swofford, 1993). Finally, the phylogenetic
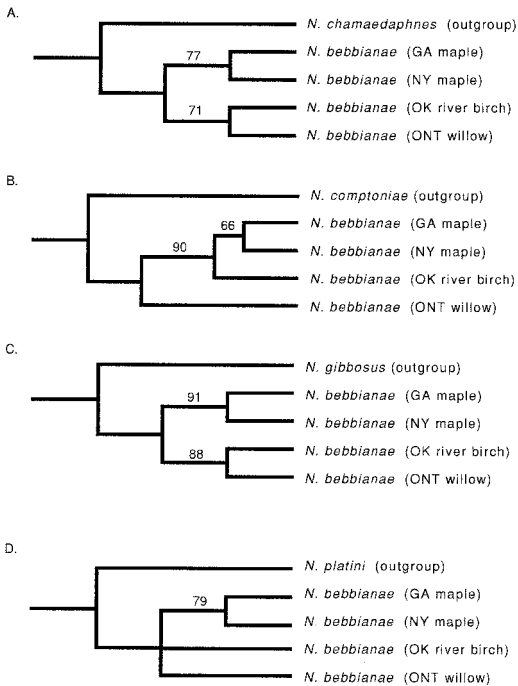
FIGURE 1. Topologies found by Wagner parsimony for four host-associated races of the *Neochlamisus bebbianae* using different outgroup species (bootstrap values based on 100 replicates are shown). Sixteen discrete characters were obtained from regressions of partial warp scores on log(centroid size) following the protocol of Fink and Zelditch (1995). A different outgroup was used for each analysis: (a) *N. chamaedaphnes* (CI = 1.000, RI = 1.000). (b) *N. comptoniae* (CI = 1.000, RI = 1.000). (c) *N. gibbosus* (CI = 1.000, RI = 1.000). (d) *N. platini* (CI = 1.000, RI = 1.000).

independence of the characters was assessed following Fink and Zelditch's (1995) protocol.

Figure 1 shows the single most parsimonious trees (MPT) from each analysis. For each MPT, none of the characters exhibiting multiple changes along the phylogeny occurred at the same nodes. Thus using Fink and Zelditch's (1995) protocol, all characters were determined to be phylogenetically independent of one another. From Figure 1, it is evident that the choice of a reference (starting) form has dramatic consequences on the resulting ingroup topology. There are several possible explanations for this. First, using the protocol of Fink and Zelditch, one codes the discrete characters of the ingroup taxa relative to the character states of the reference outgroup prior to running the phylogenetic analysis. If the relationship between size and shape is not constant between the possible reference outgroups, then changes in ingroup character states will occur. To avoid such difficulties, one usually polarizes characters by using multiple outgroups (for a discussion, see Swofford et al., 1996). Although multiple outgroups can be analyzed phylogenetically using Fink and Zelditch's protocol, only one reference outgroup can be used, as the thin-plate spline is based on only a single reference form. Therefore, the traditional solution of desensitizing characters (Swofford et al., 1996) to the choice of outgroup cannot be applied to their protocol. Second, because the principal warps are based solely on the landmark configuration of the reference specimen, choosing a different outgroup to serve as the reference specimen virtually guarantees differences in the meaning of each partial warp (for a discussion of choice of reference, see Rohlf, 1996, 1998). Therefore, the partial warp scores of the ingroup taxa are not invariant to changes in reference selection. Such lack of character state invariance is an undesirable property for data used in phylogenetic studies.

CHOOSING A BASIS FOR TANGENT SPACE

It cannot be overemphasized that the space in which the specimens reside (the space tangent to shape space) is biologically important, not the individual axes that describe the space (Bookstein, 1991; Rohlf, 1996). Because the relative distances among taxa in tangent space are of interest, any orthogonal basis spanning tangent space is equally relevant to the study of shape. This is because most statistical results (from, say, principal components analysis or multivariate analysis of variance) are invariant to rigid rotations. Therefore, one may think of rotating the space and expressing the partial warp scores in terms of a new orthogonal basis. Such a rotation does not lose any information about shape, nor does it change the relationships of taxa within the space.

If the discrete characters generated from Fink and Zelditch's (1995) protocol do not change when an alternative basis for tangent space is used, then the same ingroup tree topology will be obtained from different rotations of tangent space. This would be an important test of their protocol, for characters sensitive to arbitrary rotations of the original data space are not reliable for phylogenetic studies. We therefore took the partial warp scores from a TPS analysis of the host races of *N. bebbianae,* using *N. gibbosus* as the reference outgroup, coded the ontogenetic shape regressions following Fink and Zelditch, and generated an estimate of the phylogenetic relationships among the taxa, again using Wagner parsimony (Fig. 2a). We then chose three alternative, arbitrary bases for tangent space, found from three different rigid rotations of the partial warp scores. We coded the ontogenetic shape regressions for scores along the axes of each new basis, and reestimated the phylogeny with these newly coded characters (Figs. 2b–e). Unfortunately, we found tree topologies to be very sensitive to different alternative bases for tangent space when using their protocol. Our results show that different choices of a basis for tangent space result in different tree topologies. Because different bases are merely a rigid rotation of the partial warp scores, which loses no information about the relationships among taxa in tangent space, we must conclude that transforming the rotated continuous data (regressions of shape on size) to discrete character states loses information. Further, we must also conclude that Fink and Zelditch's protocol is very sensitive to the choice of basis for tangent space, and inconsistently retains information about the relationship between size and shape.

Only in recent years has the TPS been used to describe shape variation within and between populations (e.g., Bookstein, 1991; Zelditch et al., 1992; Swiderski, 1993; Rohlf et al., 1996; Adams and Funk, 1997). Although new applications of the thin-plate spline are constantly being investigated (e.g., Morin et al., 1995), we feel that the way Fink and Zelditch (1995) used par-
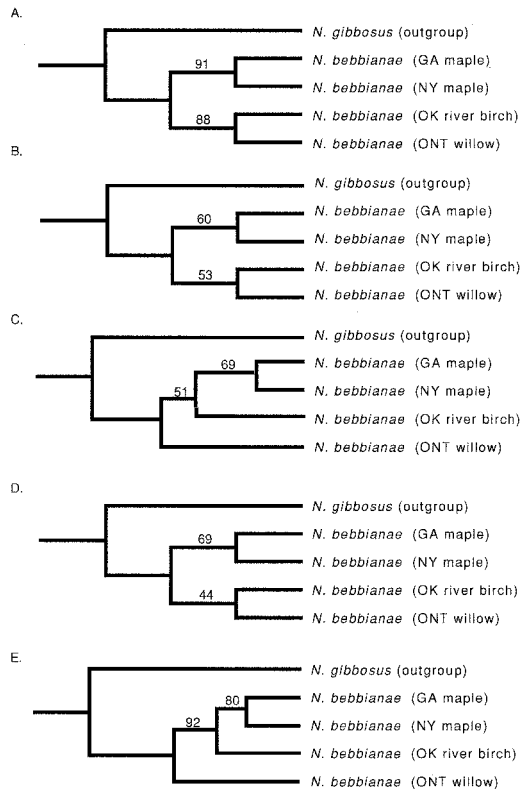

FIGURE 2. Topologies found by Wagner parsimony for four host-associated races of the *Neochlamisus bebbianae* using *N. gibbosus* as the outgroup species (bootstrap values based on 100 replicates are shown). Sixteen discrete characters were obtained from regressions of partial warp scores on log(centroid size) following the protocol of Fink and Zelditch (1995). A different basis for tangent space was used as the initial data set found from rigid rotations of the partial warp scores prior to coding discrete characters: (a) no rotation (original partial warp scores) (CI = 1.000, RI = 1.000); (b) principal components rotation (CI = 0.900, RI = 0.667); (c) and (d) QR decomposition rotation (CI = 0.875, RI = 0.600); and (e) random rigid rotation (CI = 0.889, RI = 0.714).

tial warps for phylogeny estimation has serious difficulties that must be overcome. In the discussion of their paper, they bring up several aspects of the method that warranted further examination and debate. Among these, they felt the most critical question was ''whether choosing different starting forms or even choice of an alternative basis for shape space would result in different phylogenies'' (Fink and Zelditch, 1995:358). We have shown through ex-

ample that the choice of starting form, as well as the choice of basis, has dramatic consequences on the resulting phylogeny, and therefore raises questions concerning the reliability of their coding procedure. Bridging the gap between ontogeny and phylogeny has long been a goal of evolutionary biologists. The work of Fink and Zelditch is an important step toward our understanding the relationship between these two concepts. Because of our findings, however, as well as the findings of Naylor (1996) and Rohlf (1998), we must conclude that the characters describing ontogenetic shape trajectories obtained from the protocol of Fink and Zelditch are not suitable for character-based parsimony methods.

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