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Editorial

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Will the Real Phylogeneticists Please Stand Up?*

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Abstract

In a recently published commentary, Mooi & Gill asserted that there is a crisis brewing in systematic ichthyology caused by a failure of investigators to apply the basic tenets of outgroup comparison to recover clades based solely on shared apomorphic characters. The result, they claim, is that many recent analyses disregard real synapomorphies and discover clades by phenetic rather than phylogenetic principles. We take the opportunity to refute this claim and assert that matrix-based analyses, whether parametric or nonparametric, satisfy the basic tenets of Hennig's methods, resulting in monophyletic groups confirmed by synapomorphies.

Introduction

Mooi & Gill, (2010; hereafter M&G) claim that molecular phylogenetics is not true phylogenetics but rather "phylophenetics." If true, this serious charge casts doubt on the efforts and results of all systematic studies that employ optimization methods to analyze molecular data. Before we get into specifics of the criticisms levied by M&G, we need to make it clear that the foundation of their arguments rests not on scientific rigor, but rather on opinions about the re-classification of fishes using molecular data. This bias is the reason that they only targeted researchers who proposed changes in the higher-level taxonomy of fishes using phylogenetic hypotheses based on DNA sequence data (Miya et al., 2007; Smith & Craig, 2007; Thacker, 2009). In criticizing these studies, they do not suggest any alternative relationships or provide any counter evidence to the proposed relationships. Despite the limitations of their critique, M&G would have a point if molecular systematists were truly practicing phenetics. Here we show, in no uncertain terms, why that is not the case.

The M&G thesis rests on the following four points: 1. The reliance on optimization algorithms is the modern incarnation of authority-based taxonomy. 2. Outgroup comparisons no longer play a critical role in determining character polarity. 3. The use of optimizations to build trees is not "cladistic." 4. Not showing synapomorphies on a phylogeny obscures the readers' ability to judge alternative hypotheses, and measures of node support do not refer to the quality of individual characters and their states. Instead these nodal supports refer to more abstract measures, such as bootstrap support or conditional probabilities.

We will demonstrate that each of these points is either false or irrelevant.

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M&G Claim 1: The reliance on optimization algorithms is the modern incarnation of authority-based taxonomy

Initially, cladistics overthrew 'the solutions' of the authority figures of Simpson, Romer, and Mayr. However, the refocus on solutions, the computationally 'best' result, through a reliance on computer algorithms has, perhaps, created a new set of authority figures: MrBayes and PAUP—Mooi & Gill, 2010: 29

Mooi and Gill entitled their paper "A crisis in fish systematics" because they long for the days when "real" ichthyologists found "meaningful" characters and "true" relationships. Equating "authority" to programs that run predictable and repeatable algorithms to find the best tree under a given optimality criterion or a Bayesian consensus is a dubious criticism of modern phylogenetic analyses. In fact, explicit algorithms allow researchers to reconstruct hypotheses of evolutionary relationships that move our field away from prior practices based on opinion and bias. Anyone can search for optimal phylogenetic solutions, so long as they can gather or assemble the data. If one does not agree with the limits and relationships of Thacker's (2009) gobiiforms, Smith & Craig's (2007) perciforms, or Miya et al.'s (2007) lampriforms, one can easily obtain these data and reanalyze them, or gather new data (molecular or morphological) and combine them with the original data. If one does not agree with the model employed then one can discuss reasons why it is not appropriate. If one wishes to view the synapomorphies supporting particular nodes, one need only to download the data from GenBank and perform ancestral states reconstruction for all the characters in the analyses. The availability of these characters from nearly every molecular phylogeny is a vast improvement over previous phylogenetic studies where raw data used to generate phylogenies were not available and only interpreted data were available in matrices (when published) or character descriptions/illustrations in the body of the paper.

M&G Claim 2: Outgroup comparisons no longer play a critical role in determining character polarity

Outgroup comparison no longer plays a critical role in determining which character states support a topology. This gradual erosion of the role of outgroup comparison has come about as we have moved further from an understanding of characters themselves and as shared character states, regardless of outgroup state, are seen as evidence.—Mooi & Gill, 2010: 30

Mooi and Gill make much of homology (and rightly so) because, in the absence of time machines, homology is the foundation of phylogenetic analysis. Their treatment of homology, however, is curious. Mooi and Gill state that within a transformation series the states coded "one" are apomorphic but the states coded "zero" are not equivalent among taxa. This, they state, is because the code "zero" (0) only means "not having one" (1). But is this true? Consider a column of data where the presence of pectoral fins is coded "zero" and the presence of forelimbs is coded "one." Do all taxa coded with "zeros" share a state? Of course they do, and, in fact, this state is homologous among all taxa with pectoral fins. Are pectoral fins homologous with forelimbs? Yes, given present knowledge. They are, in the sense of Wiley (2008), "transformational homologues," in that one is a property of a larger monophyletic group (Gnathostomata) that includes a smaller monophyletic group within it (Tetrapoda) that has the apomorphic property. (Naturally we would have to include a lamprey to formally polarize the transformation series). So the sweeping generalization that "…having a 0 only means 'not having 1' – which means: we have no further information" (M&G, p. 30) seems, at best, misplaced, and, at worst, a misunderstanding of homology itself. The whole point of the distinction between plesiomorphy and apomorphy is that one character (the plesiomorphy), homologous among basal members of a monophyletic group, is changed into another character (the apomorphy) in an ancestor of a less inclusive monophyletic group. Without plesiomorphic homologues, there can be no apomorphic homologues. The only time when absence is truly absence is in cases of such phenomena as gene duplication. One might argue that the fact that snakes lack limbs is evidence of true absence. While debatable (as compared to the real transformation: *presence* of a change in the developmental program to suppress limbs), no one would argue that the absence of limbs in snakes is the same as the absence of limbs in jellyfishes (Patterson, 1982). Lampreys really do not have pectoral fins, but they do have structures that will be transformed into the gnathostome pectoral girdle. (It is not as if they have a hole on each side of the body where pectoral fins would be.) Apomorphies are

transformed out of preexisting structure by informational changes that ultimately reside in the genome. After all, we are working with, we hope, heritable characters. In a matrix of inclusive monophyletic groups, "zero" should mean something. If it does not, then where is the evolution? It is also unclear what implications M&G's argument would have for characters with more than two states and the ordering of those characters.

Mooi and Gill make another curious statement when they refer to alternate character states in a DNA matrix. They claim that the alternate states at an aligned position are not homologous with the derived state. In this, they completely misunderstand molecular homology where base pairs at a particular position in the gene, as shown by the alignment, are considered homologous until proven otherwise (following Hennig's Auxilliary Criterion; Hennig, 1966). If thymine is the derived state for a group of taxa that is recovered as monophyletic on the tree, then the plesiomorphic state adenine found in its sister group certainly is not non-homologous unless evidence is presented to the contrary. It is the plesiomorphic homologue of the apomorphic state. Naturally, if the position is noisy, not all the thymines (or other base residues) need be uniquely derived; some states might be homoplastic, but this is a conclusion drawn *a posteriori* after accepting one particular tree over another (a manifestation of reciprocal illumination among the data at hand). Further, if the distribution of thymine on a tree does indicate homoplasy, then it is possible that two or more subclades have the synapomorphic property "thymine present." What is homoplastic at one level (the entire transformation series) may be locally synapomorphic (i.e, homoplasy at one level may be homology at another; otherwise, synapomorphies would always have a consistency index of 1). That was the point of Källersjö et al. (1999), who certainly did not advocate grouping by homoplasy (a claim made by M&G). The simple, obvious physiological example is homeothermy in birds and mammals. Homeothermy is homoplastic in its distribution among amniotes, but independently synapomorphic for the clades Mammalia and Aves.

Mooi and Gill's criticism is even more problematic when they frequently use losses to diagnose clades in their empirical work. For example, Mooi & Gill (2004) coded 14 of 59 characters (3, 4, 12, 26, 27, 28, 32, 33, 35, 36, 38, 46, 51, and 55) with a state of "one" for secondarily lost features. If the data from this study were expanded to more inclusive clades, the coding strategy argued for by M&G would dictate that the primitive absence of a feature needed to be characterized as M&G's proclaimed non-homologous state "zero." In the work cited above, this would unite Plesiopidae and several more restricted clades within the family by the state "zero," synapomorphies that M&G eschew and blame on optimization procedures. These changes are not due to optimization procedures, but are, instead, a by-product of taxonomic scale. Mooi and Gill's various studies are usually focused on Johnson & Patterson's (1993:555) "disparate twigs of the [percomorph] tree," whereas the explicit studies they criticize are large-scale and taxon-rich datasets that have not otherwise been analyzed in Percomorpha. At large scales, homoplasy is rampant in both morphological and molecular characters, and the crude set-theory-based procedures using presence-absence coding embraced by M&G and discussed in Ebach et al. (2008) are guaranteed to fail without computer-aided optimization procedures. Further, these set-theory/presence-absence methods, as employed by M&G in their analyses, rely on previous anatomical work that allows them to determine character polarity, seemingly without transformations. Never mind that this procedure is assumption laden when compared to simply including real outgroups with real characters; this appeal to the undoubtedly noble work of past morphologists is neither possible nor desirable for DNA sequence data. There is no baüplan for a vertebrate histone H3 DNA sequence, and, even if there were, the loss of information in coding it with states "one" and "not having one" would result in an inferior, imprecise, and suboptimal hypothesis of relationships. Without large-scale, explicit analyses, we will fail to resolve the percomorph bush.

One final character issue demands discussion. Mooi and Gill make much out of "using biology, not algorithms to make homology decisions." Who but the careless or untrained investigator does not use biology to reach decisions on homology? All methods depend on the intelligent formation of a transformation series (a column of data). Each transformation series, if formed correctly, should have passed initial tests that lead one to assume that the characters are indeed matches (Sober, 1988), also known as primary homology hypotheses (DePinna, 1991) or initial hypotheses of homology (Wiley, 1975, 1981). That is, one assumes they have passed the tests of similarity (various tests of Remane, 1956) and conjunction (as summarized by Patterson, 1982, 1988). Such tests are available for both morphological and molecular characters. To construct a matrix of DNA bases or amino acids (or gene order) we use all the tools of gene identity (to avoid paralogy when possible), alignment of bases within a gene, and even thermodynamics (e.g., when alignments of bases in ribosomal genes are made relative to secondary structure). But the final arbiter of initial matches that share the same identity (e.g., the same initial coding or naming) is, and will always remain, in the phylogenetic system, congruence (Hennig, 1966; Patterson, 1982). Congruence is simply

another manifestation of Hennig's concept of reciprocal illumination, pitting one hypothesis of synapomorphy against another in the arena of what is now called "optimization." Thus, this area of primary concern for M&G is clearly misplaced. All methods depend on the skill and experience of the investigator to form transformation series (data columns in a matrix) with robust hypotheses of initial homology statements using solid biological criteria. Mooi and Gill may not accept that we can be informed about molecular data through biology, but the fact remains that we know a great deal about the evolution of genotype, perhaps more so than phenotype.

Now that we have discussed issues of character homology, three questions thus arise regarding the use of outgroups in modern phylogenetic analyses: 1. Do the commonly published molecular phylogenies use outgroups? 2. Are outgroups used to polarize characters in the resulting tree? 3. If outgroups are used effectively to polarize characters, where are those synapomorphies? After all, without outgroups and synapomorphies, M&G (2010) may have a point.

We picked some studies among recent molecular-based papers to demonstrate that they incorporate outgroups. Kawahara et al. (2008) used two basal outgroups, *Polymixia japonica* and *Beryx splendens* (as well as a plethora of percomorph outgroups) to test the relationships within gasterosteiform groups. Miya et al.'s (2003) "higher teleost" phylogenetic analysis also included basal outgroups from the sister group of euteleosts, the herrings and minnows. Our own analyses also used basal outgroups (e.g., Wiley et al., 2000; Miya et al., 2007; Smith & Craig, 2007; Thacker, 2009). Therefore, the charge that outgroups are disregarded by phylogeneticists using molecular data is simply untrue as a matter of published record.

The trickier question is not whether or not molecular studies use outgroups, but whether those outgroups are used to polarize, *a posteriori*, characters such that putative monophyletic groups are confirmed by putative synapomorphies. There are two possible answers: 1. No, the groups resolved are grouped by overall similarity, with outgroups only being a garnish (the result being phenetic groups, "following the argumentation of Sokal and Sneath [1963]"). 2. Yes, the groups resolved are groups corroborated by synapomorphy. If the answer is yes, then the objection lies in a failure to map the synapomorphies rather than a lack of synapomorphies, which we will discuss in our analysis of Claim 4.

M&G Claim 3: The use of optimizations to build trees is not "cladistic"

With the optimization approach, topologies will change with the addition of taxa as the combination of character states changes and new optimal arrangements result—what were once homologies become homoplasies and vice versa. If we understand character homology, inserting a new taxon should generally not influence the relative relationships of the original taxa. When topologies change with the addition of taxa, this is an indication that there is a misunderstanding of character homology.—Mooi & Gill, 2010: 30

If the homology of characters is identified correctly, the insertion of a forth taxon into Nelson's (2004) set of three, above, should not alter the relative positions of the initial taxa – elegant, logical in an evolutionary sense, and stable—Mooi & Gill, 2010: 27

The idea that it is an inherent property of "optimality" approaches to always change topology with the addition of new taxa or characters is a sweeping assertion that is not based on first principles. The fact is, there is no reason why using "optimality" approaches should inherently lead to unstable trees or changing hypotheses of homology because, as we shall see below, "optimality" approaches are not only phylogenetic but are even Hennigian. There may be many reasons for unstable hypotheses, but the reasons for this instability do not lay in the methods but rather in the data and the taxa included in a particular study. Yes, of course when topologies change with the addition of data one may have indeed misunderstood a character match. But to not acknowledge that one can make mistakes requires error-free science. Of course we wish there was a way to determine *a priori* that all of our matches were true homologies. Alas, we know no way of doing so with molecular *or* morphological information. These statements by M&G imply an incomplete understanding of, or a disregard for, modern phylogenetic algorithms, never mind the tentative nature of science in general. Inferred relationships, homology hypotheses, and character optimizations must always be reassessed after the addition of new data. All reasonable researchers should expect some hypotheses to improve as additional morphological or molecular data are collected and analyzed.

In the hypothetical three-taxon statements M&G discuss, the addition of one taxon and the concomitant refinements to all comparative hypotheses should occasionally change the relationships; similarly, the addition of new characters could result in similar changes. Mooi and Gill's expectation that new data will not change any hypotheses of relationships is rigid, dogmatic and assumes perfect knowledge. Optimization, as we shall see below, is a process common to all computer-aided phylogenetic analyses, and, in fact, is also inherent when character conflict is encountered in Hennigian argumentation.

One of the major issues that concern M&G is that in "most molecular presentations, optimization is used to build trees" (M&G, pg. 30). We find their concern with optimization disconcerting; all modern methods of phylogeny reconstruction, including parsimony (nonparametric) and statistical (parametric) approaches, use some form of optimization to sort through trees, regardless of whether the data involved is molecular or morphological. In fact, very little effort is expended in actually "building" trees using parsimony or statistical methods. As a summary, there are four methods of phylogenetic analyses that do not rely on phenetic (distance estimates) methodology: (1) classical Hennigian argumentation, (2) parsimony analysis, (3) likelihood analysis, and (4) Bayesian inference. Of the four, Hennig is known to us only to have used Hennigian argumentation. At least one of us (EOW) is old enough to have also used classical Hennigian argumentation in publication (Wiley, 1976) and can speak directly to why he began using parsimony algorithms as an alternative: Parsimony algorithms mimic classical Hennigian argumentation. They are simply more efficient, especially in the face of conflicting data. Surely M&G are not calling for a return to classical Hennigian argumentation as the *only* general method of phylogenetic analysis. Below we briefly contrast the parsimony method with Hennigian argumentation as an example of how optimizations are indeed "cladistic." We use "cladistic" in the sense that a distinction is made between symplesiomorphy and synapomorphy, and this difference is identified via phylogenetic analysis that ultimately circumscribes clades confirmed (as hypotheses relative to the data at hand) by synapomorphies.

Going from the data to a tree or set of trees, one polarizes characters by maximizing homology and explanatory power and, we hope, minimizing homoplasy in reference to one or more outgroups. Hennigian argumentation depends on *a priori* polarization of characters, and trees are built transformation series by transformation series. For a classic Hennigian analysis, the greater the burden of homoplasy, the greater the number of possible alternative phylogenetic trees to consider, and the more subject the results of the analysis becomes to things like starting points. This may be the primary reason phylogeneticists moved from Hennig argumentation to parsimony algorithms.

Modern parsimony algorithms handle characters somewhat differently. Parsimony algorithms do indeed optimize characters on unrooted trees; this is necessary in order to determine the length of the tree and thus compare lengths of different trees. The assumption that allows us to work with unrooted trees is the assumption of free reversibility. This does not mean that characters reverse willy-nilly, but they are allowed to reverse if the weight of evidence provided by the distributions of other characters, indicates that a reversal is in order (i.e., the resulting tree is shorter). While it is true that you can build trees using a parsimony algorithm (e.g.,Wiley, 1981 and Wiley et al., 1991), most algorithms spend their time evaluating trees by doing such machinations as branch swapping in an effort to find better trees, with "better" being defined as shorter given the data and model. Likewise, parametric methods also spend most of their time performing machinations in an effort to find better trees, with "better" being defined as more likely given the data and model. (Bayesian analyses works a bit differently, but the difference isn't relevant to this particular point.). Does this make them "un-Hennigian" or "non-cladistic?" No, it does not. If an outgroup is used in the analysis, that outgroup is used to provide direction to the unrooted tree and the result is grouping by synapomorphy. The fact that one optimizes character distributions on an unrooted tree or on many unrooted trees is not in any way contrary to the principle that outgroups should be used to polarize characters, nor does optimization on an unrooted tree mean that synapomorphies have disappeared. In parsimony, one can "map" the synapomorphies easily and they appear to be the same synapomorphies that one would obtain if one performed classical Hennigian argumentation with *a priori* character polarization (at least on those matrices that are simple enough to follow the argumentation). In other words, the major difference between *a priori* versus *a posteriori* character polarization is computational efficiency. The result (a tree or trees with synapomorphies) is the same. That is the major reason why "classical Hennigians" made nice with "classical Wagnerians" in the 1970s. To complain that we have lost our way because optimization is used to build trees is wrong. Mooi and Gill are confusing the fitting of initial homology statements on an unrooted tree, and *a posteriori* polarization in reference to a designated outgroup, with the process of building of a rooted tree by Hennig argumentation by *a priori* polarization. As

we shall demonstrate below, the same is true of parametric analyses; that is, outgroups effectively polarize unrooted trees to yield synapomorphies. Times have changed: although the intimate process of setting polarity in a matrix with one outgroup on a small taxon set with pen and paper is what we all learned, we all have moved on to larger data matrices that require the use of algorithms and optimizations.

M&G Claim 4. Not showing synapomorphies on a phylogeny obscures the readers' ability to judge alternative hypotheses, and measures of node support do not refer to the quality of individual characters and their states. Instead these supports refer to more abstract measures, such as bootstrap support or conditional probabilities

Statistics such as jackknife resampling percentages, bootstrap values, and posterior probabilities do not allow fruitful comparison among competing hypotheses, only homologies do. It might be argued that the statistics are shorthand for the characters that support the tree. Unfortunately, no one can read the shorthand.—Mooi and Gill, 2010: 38

Mooi and Gill are correct that the results of molecular analyses are rarely expressed as synapomorphies on trees or for nodes (see Sparks & Smith, 2004 and Frost et al., 2006 for exceptions). But the synapomorphies are, in fact, present. It is true that not mapping synapomorphies on the tree makes comparing the original data matrix with the results less transparent. But the connection can be demonstrated using ancestral states reconstruction of actual nucleotides on actual branches forming actual synapomorphies. At worst, the phylogeneticists M&G criticize would be guilty of ignoring the utility of showing synapmorphies on an otherwise robust (or weak) result. However, if the synapomorphies are present, molecular phylogeneticists cannot justly be accused of being pheneticists. In other words, if the synapomorphies are really present but not mapped, then the problem is not a lack of synapomorphies but a lack of appreciation for the fact that showing the synapomorphies to the satisfaction of a third party might be a good idea. (The question of whether the third party can reasonably demand such mapping is another matter.)

Synapomorphies can be retrieved from any "modern" parametric analysis through the ancestral states reconstruction (Schluter et al., 1997; Pagel, 1999) in software packages such as Mesquite (Maddision & Maddison, 2009). Unlike more global measures of character performance (e.g., consistency or retention indices), ancestral states reconstruction attempts to reconstruct the character state at a particular node, not measure its performance over the entire matrix. If a simple model is employed in a Bayesian framework, the result is the posterior probability of the state conditional on the tree topology and associated model parameters. If in a likelihood model, they are likelihood estimates. This provides the opportunity to determine if a state is synapomorphic. So, let us examine what happens when we do this with morphological and molecular data. First, we will examine a morphological dataset to address the question that parametric statistical methods are actually grouping by "phylophenetics," which would result in clades not marked by synapomorphy, but rather overall similarity. After considering the morphological case, we will turn to a molecular example.

The morphological analysis is based on the classic Gauthier et al. (1988) matrix of amniote relationships, including both fossil and living amniotes. It has all of the characteristics we require, including missing data, homoplasy (analyzing only the living taxa results in birds+mammals as a clade), and plenty of data columns. We assume, but do not test, the proposition that Gauthier and colleagues formed their initial hypotheses of homology in a reasonable, "biological," and defensible manner. Parsimony analysis of the matrix results in a single tree using PAUP* (Swofford, 2002) with TBR, 100 random addition searches and equal character weighting that is identical with the original result of Gauthier et al. (1988). A Bayesian analysis results in a consensus tree of identical topology using the MK model of Lewis (2001) and the statistical support for each node in the tree was 95% or greater. To see where the synapomorphies were found in the parsimony tree, we optimized the character states using DELTRAN. To see if there were any synapomorphies in the Bayesian analysis, we implemented the likelihood and parsimony ancestral state reconstruction routines in Mesquite (Maddison & Maddison, 2009). Unfortunately ancestral states reconstruction cannot be performed for data columns with missing data, so we were only able to perform reconstruction on the 207 hard anatomical characters. Of the 198 unambiguous synapomorphies that appeared on the parsimony tree, all 198 appeared on the same nodes in the MrBayes tree. The only difference was that the likelihood probabilities were on the order of 95-99% while those on the parsimony tree were 100% (CI=1.0). Charac-

ters that were ambiguous on the parsimony tree (9 of 207) were also ambiguous on the same nodes in the likelihood analysis, and there were no character columns that were totally different in their interpretations.

To address the specific claim that molecular analyses do not group by synapomorphy we asked Mark Holder (University of Kansas) if he would extract the change lists from the analysis of Miya et al. (2007) using a script in the DendroPY package (Sukumaran & Holder, 2010). The grouping *Stylephorus* + *Merluccius* is associated with eight (8) matches that can be interpreted as synapomorphies and have a posterior probability conditional on the model used in the original analysis and the tree topology of 80% or greater, with two of these synapomorphies having values of 95%. The probabilities of not changing varied from 3% to 13%. This should not be interpreted in the frequentist mode; there is no 95% threshold of statistical significance. Rather it is simply a Bayesian probability. These same eight (8) synapomorphies had similar likelihood probabilities when the character states were reconstructed in Mesquite. The grouping *Percopsis* + (*Stylephorus* + *Merluccius*) had only four matches of this quality, with these nodes recovering more ambiguous probability support for specific synapomorphies under the likelihood reconstruction (50-76%), but that is to be expected as the posterior probability of this particular node was 90% rather than 100%. Therefore, in this example, at least, there is a positive correlation between node support and underlying synapomorphy support with the *Stylephorus* + *Merluccius* clade possessing a number of unambiguous character synapomorphies and strong statistical node support while the *Percopsis* + (*Stylephorus* + *Merluccius*) clade had weaker node support and the character synapomorphies are more ambiguous.

Of course, these are only two analyses. No doubt matrices with greater amounts of homoplasy, instances of long branch attraction, instances of long branch repulsion, etc., may make a parsimony tree different than a MrBayes tree or may result in very weak support in term of the posterior probabilities of an ancestral states reconstruction at a node. However, these demonstrations reveal that sweeping generalizations that statistical approaches to phylogeny reconstruction are not based on synapomorphies are simply untrue. The Bayesian analyses resulted in monophyletic groups confirmed by synapomorphies, and the placement of these synapomorphies in the case of the amniote analysis was identical to the placement of synapomorphies in a parsimony context and, we would assume, a traditional Hennigian argumentation analysis. Thus, to conclude that statistical approaches are not phylogenetic *sensu* Hennig is a claim that can be refuted empirically. The claim that "Optimization does not differentiate between homology and nonhomology…" (M&G, p.38) is without merit. Additionally a larger question remains unknown: what is the connection between support values such as the posterior probability of a node and the underlying synapomorphic support for a much larger sample of analyses? Are all nodes with high posterior probabilities always corroborated by one or more character changes that also have a high synapomorphic probability? We don't know, but then again, are all nodes in a morphological analysis always supported by unambiguous unique and unreversed morphological synapomorpies? The answer, of course, is no.

Why is ancestral states reconstruction not typically employed by molecular systematists? We can only give our opinions. It is a labor-intensive operation in its present stage of development that is normally employed for study of a few characters relative to a phylogeny, and it is difficult to compare studies because alignment variation from study to study makes analysis-specific nucleotide positions non-comparable (e.g., site 32 in study A might be site 350 in study B. (Note that they are still homologous sites, but in different analyses employing different amounts of data and possibly at different levels of universality.) It is not so automatic as spitting out a list of synapomorphies from a parsimony analysis in PAUP*. Another reason for this is no doubt due to the nature of molecular characters themselves in terms of discussing character evolution. DNA data comes in five classes (i.e., the four bases and indels), and the homology of such data depends on sequence alignment, not the intrinsic qualities of the structure of the molecules. A thymine is a thymine is a thymine even if all are not homologous because they are found in different data columns. All thymine share an identity as a class, but not all thymines are phylogenetically homologous (for discussion of identity and homology see Wiley, 2008). This makes thymine rather uninteresting to a morphologist who is worried about whether the first epural of an *Esox* is homologous with the first epural of an *Elops*. It also does not leave much to discuss by a molecular systematist. What are you going to say about thymine except that the presence of thymine at position 619 of RAG1 corroborates the monophyly of group *Stylephorus* + *Merluccius* with a likelihood probability of 95.7% while the probability of no change is only 3.2%?

We do hope for a robust discussion of molecular character evolution in the near future. Identifying the molecular synapomorphies supporting clades will lead to clarity of the underlying node support, and potentially new information regarding the molecular evolution of a clade that has previously gone unreported (e.g., clades that share large base pair insertion or deletion events), and it is not unreasonable for M&G to ask for this information. How-

ever, there is yet another problem. Mooi and Gill complain that the robustness of the evidence is not manifest in molecular trees in a form that makes the results directly comparable to those derived from a morphological analysis. This complaint is irrelevant and only has merit if the investigating scientists fail to understand what the "shorthand" represents; in this case "shorthand" describing the seemingly abstract language of posterior probabilities or bootstrap values. There are numerous works that detail the scientific and statistical information contained in these values and how they should be properly interpreted (e.g., Felsenstein, 1985; Efron et al., 1996; Erixon et al, 2003; Felsenstein, 2004). While it is true that measures of node support (e.g., posterior probabilities, bootstrap values) do not provide a direct, visible, link between the results and the original data (such as "base pair A transitions to G at site 784 at node 13"), to suggest that they have no descriptive or comparative value regarding node support is positively misleading and uniformed. The truth is quite the opposite and a failure in understanding the "shorthand" is not the same as a failure in the "shorthand" to provide a descriptive assessment of a clade's support. Additionally, if a researcher is interested in comparing different phylogenetic hypotheses for a clade, whether two molecular hypotheses or a molecular and morphological reconstruction, there is an abundance of hypothesis testing literature available as well (e.g., Huelsenbeck et al., 1997; Shimodaira, 2002). One does not have to rely solely on node support values or synapomorphy distributions to assess and test the "quality" of competing hypotheses of phylogenetic relationships.

Summary Remarks

In the end, we are left wondering what M&G will argue against as we progress into whole-genome phylogenetic analyses or evo-devo studies that challenge fundamental assumptions of morphological studies. Our understanding of, and interest in, the phenotype will only increase as we learn more about the genetic basis of morphological change. Our focus on detailed morphology will not decrease, it will increase. Mooi and Gill make an argument for a return to a focus on character analysis instead of searching for the best trees. We argue that cladistic analyses have always been about finding the best trees using evolutionarily informative characters and thus the focus has always been on character analysis. The process of discovering characters, understanding homology, and identifying synapomorphy and homoplasy are the similar endeavors at different stages in a phylogenetic study. It is clear that there is room under the phylogenetic tent for many different approaches to phylogenetic systematics *sensu* Hennig (1966), just as there is room for many different kinds of character evidence. We see these connections when we analyze relatively simple problems regardless of the phylogenetic method. That is, if the topologies are identical regardless of the particular method employed and rooted in the same manner, why should we expect the synapomorphies to be distributed in a different manner? The results are what Hennig (1966) would recognize as phylogenetic: monophyletic groups confirmed and corroborated by synapomorphies. The more conflict in the data, the more tools we need to employ to sort out the mess. Different methods have different advantages. Among the modern methods, parsimony is fast, and if one maps synapomorphies, very transparent relative to the original data. However, many systematists want to take advantage of what we have learned about molecular evolution and incorporate that knowledge into model based approaches. Likelihood, Bayesian and weighted parsimony use more explicit models of character change than equally-weighted parsimony analysis, but at least in likelihood and MrBayes analyses the best-fit model is employed. Pick a model that fits the data poorly (or weights in a bizarre manner) and the results will be problematic. Parsimony and likelihood provide point estimates; they find the best tree under their respective criteria. Differentiating among trees is a relatively simple proposition in likelihood analysis using a well-understood statistical test that can be employed during the optimization machinations. Parsimony simply tries to find the shortest tree and what the model might be is continuous (Goloboff, 2003). A MrBayes (Huelsenbeck & Ronquist, 2001) analysis uses likelihood models but provides for an estimate of uncertainty since the results are not a single fitted model (a point estimate) but a population of fitted models, giving the investigator some idea of the overall uncertainty of the data relative to all trees without the need to bootstrap the results. One could argue that all methods are logically "Bayesian" if outgroups (prior knowledge) are used to affect results (polarization resulting in synapomorphy).

Should those who perform molecular analyses consider alternative morphological hypotheses? We hope they will. Until recently, much of that evidence was scattered. With the appearance of the synthesis of Wiley and Johnson (2010), the job of considering (or at least calling attention to) the alternative morphological evidence for fish

groups at higher taxonomic levels is made easier. Should morphologists consider alternative molecular evidence? Certainly, and one route, if they wish to do so, is ancestral states reconstruction. Are eight molecular synapomorphies worth more than one morphological synapomorphy? Are two morphological characters worth more than one morphological character? How about six versus five? None of us can answer those questions, but we suspect that, if we continue to cut the Gordian Knot of fish genealogy as a community of united systematic ichthyologists (using both morphology and molecules) rather than as bands of warring tribes, reasonable answers will emerge.

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References

Ebach, M.C., Morrone, J.J. & Williams, D.M. (2008) A new cladistics of cladists. *Biological Philosophy*, 23, 153–156.

Efron, B., Halloran, E. & Holmes, S. (1996) Bootstrap confidence levels for phylogentic analysis. *Proceedings of the National Academy of Sciences USA*, 93, 7085–7090.

Erixon, P., Svennblad, B., Britton, T. & Oxelman, B. (2003) Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. *Systematic Biology*, 52, 665–673.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.

Felsenstein, J. (2004) Inferring phylogenies. Sinauer Associates, Sunderland, Mass.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S. C., Raxworth, C.J., Campbell, J.A., Blotto, B., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. & Wheeler, W.C. (2006) The amphibian tree of life. *Bulletin of the American Museum of Natural History*, 297, 1–370.

Gauthier, J.A., Kluge, A. & Rowe, T. (1988) Amniote phylogeny and the importance of fossils. *Cladistics*, 4, 105–209.

Goloboff, P.A. (2003) Parsimony, likelihood, and simplicity. *Cladistics*, 19, 91–103.

Hennig, W. (1966) Phylogenetic Systematics. University of Illinois Press, Urbana, Illinois.

Huelsenbeck, J.P., Rannala, B. & Yang, Z. (1997) Statistical test of host-parasite cospeciation. *Evolution*, 51, 410–419.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755.

Johnson, G.D. & Patternson, C. (1993) Percomorph phylogeny: A survey of acanthomorphs and a new proposal. *Bulletin of Marine Science*, 52, 554–626.

Källersjö, M., Albert, V.A. & Farris, J.S. (1999) Homoplasy increases phylogenetic structure. *Cladistics*, 15, 91–93.

Kawahara, R, Miya, M., Mabuchi, K., Lavoué S., Inoue, J.G., Satoh, T.P., Kawaguchi, A. & Nishida, M. (2008) Interrelationships of the 11 gasterosteiform families (sticklebacks, pipefishes, and their relatives): A new perspective based on whole mitogenome sequences from 75 higher teleosts. *Molecular Phylogenetics and Evolution*, 46, 224–236.

Lewis, P.O. (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, 50, 913–925.

Maddison, W.P. & Maddison, D.R. (2009) Mesquite: a modular system for evolutionary analysis. Version 2.72 http://mesquiteproject.org

Miya, M., Holcroft, N.I., Satoh, T.P., Yamaguchi, M., Nishida, M. & Wiley, E.O. (2007) Mitochondrial genome and a nuclear gene indicate a novel phylogenetic position of deep-sea tube-eye fish (Stylephoridae). *Ichthyological Research*, 54, 323–332.

Miya, M., Takeshima, H., Endo, H., Ishiguro, N.B., Inoue, J.G., Mukai, T., Satoh, T.P., Yamaguchi, M., Kawaguchi, A., Mabuchi, K., Shirai, S. & Nishida, M. (2003) Major patterns of higher teleostean phylogenies: a new perspective based on 100 complete mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 26, 121–138.

Mooi, R.D. & Gill, A.C. (2004) Notograptidae, sister to Acanthoplesiops Regan (Teleostei: Plesiopidae: Acanthoclininae), with comments on biogeography, diet and morphological convergence with Congrogadinae (Teleostei: Pseudochromidae). *Zoological Journal of the Linnean Society*, 141, 179–205.

Mooi, R.D. & Gill, A.C. (2010) Phylogenetics without synapomorphies — A crisis in fish systematics: time to show some character. *Zootaxa*, 2450, 26–40.

Pagel, M. (1999) The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology*, 48, 612–622.

Patterson, C. (1982) Morphological characters and homology, p. 21–74. In: Problems of Phylogenetic Reconstruction. K. A.

Joysey and A. E. Friday (eds.). Systematic Association Special Volume Number 25. Academic Press, London.
Patterson, C. (1988) Homology in classical and molecular biology. *Molecular Biology and Evolution*, 5, 603–625.
dePinna, M.G.G. (1991) Concepts and tests of homology in the cladistic paradigm. *Cladistics*, 7, 367–394.
Remane, A. (1956) Die Grundlagen des natürlichen Systems der vergleichenden Anatomie und Phylogenetik 2. Auflage, Geest und Portig K. G. Leipzig, Germany.
Schluter, D., Price, T., Mooers, A.O. & Ludwig, D. (1997) Likelihood of ancestor states in adaptive radiation. *Evolution*, 51, 1699–1711.
Shimodaira, H. (2002) An approximately unbiased test of phylogenetic tree selection. *Systematic Biology*, 51, 492–508.
Smith, W.L. & Craig, M.T. (2007) Casting the percomorph net widely: the importance of broad taxonomic sampling in the search for the placement of serranid and percid fishes. *Copeia*, 2007, 35–55.
Sober, E. (1988) Reconstructing the Past: Parsimony, Evolution, and Inference. MIT Press. Cambridge, MA.
Sokal, R.R. & Sneath, P.H.A. (1963) Principles of numerical taxonomy. W. H. Freeman, San Francisco.
Sparks, J.S. & Smith, W.L. (2004) Phylogeny and biogeography of cichlid fishes (Teleostei: Perciformes: Cichlidae). *Cladistics*, 20, 501–517.
Sukumaran, J. & Holder, M.T. (2010) DendroPY: A python library for phylogenetic computing. *Bioinformatics*, advanced access, 25 April, 2010. (http://bioinformatics.oxfordjournals.org/cgi/reprint/btq228v1)
Swofford, D.L. (2002) PAUP*: Phylogenetic analysis using parsimony (and other methods), Sinauer Associates, Sunderland, Mass.
Thacker, C.E. (2009) Phylogeny of Gobioidei and placement within Acanthomorpha with a new classification and investigation of diversification and character evolution. *Copeia*, 2009, 93–104.
Wiley, E.O. (1975) Karl R. Popper, systematics and classification: A reply to Walter Bock and other evolutionary taxonomists. *Systematic Zoology*, 24, 233–243.
Wiley, E.O. (1976) The phylogeny and biogeography of fossil and recent gars (Actinoptergii: Lepisosteidae). Miscellaneous Publications, Museum of Natural History, University of Kansas, 64, 1–111.
Wiley, E.O. (1981) Phylogenetics. The Theory and Practice of Phylogenetic Systematics. Wiley-Interscience, New York.
Wiley, E.O. (2008) Homology, identity and transformation, p. 9--21. IN: Mesozoic Fishes 4 – Homology and Phylogeny. G. Arratia and H.-P. Schultze (eds.). Verlag Dr. Friedrich Pfeil, München, Germany.
Wiley, E.O., Dimminck, W. & Johnson, G.D. (2000) The interrelationships of acanthomorph fishes: a total evidence approach using molecular and morphological data. *Biochemical Systematics and Ecology*, 28, 319–350.
Wiley, E.O. & Johnson, G.D. (2010) A teleost classification based on monophyletic groups, p. 123–182. IN: Origin and Phylogenetic Interrelationships of Teleosts. J.S. Nelson, H.-P. Schultze, and M.V.H. Wilson (eds.). Verlag Dr. Friedrich Pfeil, München, Germany.
Wiley, E.O., Siegel-Causey, D., Brooks, D.R. & Funk, V.A. (1991) The compleat cladist: A primer of phylogenetic procedures. *The University of Kansas Museum of Natural History Special Publication*, 19, 1–158.