



Dating nodes on molecular phylogenies: a critique of molecular biogeography

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Abstract

Taxa have been dated using three methods: equating their age with the age of the oldest known fossil, with the age of strata the taxa are endemic to, and with the age of paleogeographic events. All three methods have been adopted as methods of dating nodes in molecular phylogenies. The first method has been the most popular, but both this and the second method involve serious difficulties. Studies often, correctly, introduce oldest known fossils as providing minimum ages for divergences. However, in the actual analyses these ages, and ages derived from them, are often treated as absolute ages and earlier geological events are deemed irrelevant to the phylogeny. In fact, only younger geological events can be irrelevant. Studies correlating the age of nodes with age of volcanic islands often overlook the fact that these islands have been produced at subduction zones or hot spots where small, individually ephemeral islands are constantly being produced and disappearing, and a metapopulation can survive indefinitely. Correlating the age of taxa with that of associated paleogeographic events is probably the most promising method but has often been used in a simplistic way, for example in assuming that all divergence across the Isthmus of Panama dates to its final rise. Most workers now agree that a global molecular clock does not exist, and that rates can change between lineages and within a lineage over time. New methods of estimating branch lengths do not assume a strict clock, but the number of models for molecular evolution is then effectively infinite. Problems with calibrating the nodes, as well as with substitution models, mean that phylogeography's claim to be able to test between vicariance and dispersal is not justified.

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“...a great many things which have been conclusively demonstrated by the Ancients are unintelligible to the bulk of the Moderns owing to their ignorance—nay, by reason of their laziness, they will not even make an effort to comprehend them...”. (Galen, 1916).

Many recent conclusions in systematics and biogeography have been based on phylogenetic trees in which the nodes are dated. These studies have been fundamental to the whole enterprise of phylogeography and in particular its claims to be able to test between vicariance and dispersal. However, while much more attention has been paid to methodological details of phylogenetic tree construction, several crucial assumptions made in this approach, notably methods of calibrating divergence times, have remained largely unexamined.

Methods of dating evolution

Since the beginning of evolutionary biology as a science, taxa have been dated using three methods. Taxon age has been equated with the age of the oldest known fossil of the group (or a related group), with the age of strata the taxa are endemic to, and with the age of putatively relevant paleogeographic events.

Correlating the age of taxa with the age of the oldest known fossil

Dating evolutionary events has relied most often on fossils. Since Matthew's (1915) epochal work, studies of phylogeny, biogeography and evolution have attributed special significance to the fossil record. Although Darwin emphasized its fragmentary nature, Matthew (1915) favored a literal reading of the fossil record, with

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a taxon taken to be the same age as its oldest known fossil or, often, the oldest fossil of a closely related group. The absence of earlier fossils is regarded as significant. This approach was adopted by the influential New York school of zoogeography, which was based on Matthew's work and included evolutionists such as Simpson, Mayr, and Darlington (Nelson and Ladiges, 2001). However, when even a paleontologist (Gould, 1989) can cite "the most treacherous kind of argument that a scientist can ever use—negative evidence", it would seem that the "direct" paleontological method for dating the origin of taxa might have unstable foundations.

Occasionally, doubts about the method and its results are expressed. For example, based on the stunningly preserved fossils of Messel, Germany, G. Storch (quoted in Hoffmann, 2000) proposed that "Bats were already advanced 49 million years ago. I'm convinced they originated much earlier than you read in textbooks." Likewise, Ernst and Barbour (1989) wrote that the Pleurodira turtles are considered by many experts to be more primitive than the Cryptodira, but 'surprisingly' appear in the fossil record an entire period (about 50 million years) later than the cryptodirans.

New discoveries of fossils much older than the previously known oldest members of a group are made regularly. For example, until recently fossil loriform primates were previously known back to 20 Ma, but fossils dated at 41–37 Ma old have now been reported (Seiffert, Simons and Attia, 2003). Crown-group salamanders were recently found in Middle Jurassic rocks, predating the previous record by some 100 Myr (Gao and Shubin, 2003). Metatheria (marsupials and their relatives) were known back to 75 Ma ago, but a fossil dated at 125 Ma has been described by Luo et al. (2003). Hummingbird fossils were known back to 1 Ma, but recently one dated at 30 Ma was found (Mayr, 2004). These new "oldest fossils" are often regarded as highly significant and reported in prestigious journals, and their location assumed to represent a new center of origin. For Matthewians, the metatherian fossil represents "a rich source of new information about the time and place of origin" of the group (Cifelli and Davis, 2003) and "the classic views" (i.e., the Matthewian account) of the origin of marsupials in the northern continents are "corroborated" (Luo et al., 2003).

The major problems with the Matthewian approach are still usually ignored and there is even a recent book titled *The Adequacy of the Fossil Record* (Donovan and Paul, 1998a). The title is probably a reference to Darwin's (1971) chapter "On the imperfection of the geological record". In the introduction to their book, Donovan and Paul (1998b) referred to Darwin's "bias" in his well-known argument that the fossil record must be very incomplete, but it is possibly Donovan and Paul, not Darwin, who are

biased. A member of the New York school (Darlington, 1957, p. 320) has proposed that the fossil record "allows an almost magical view into the past" and Briggs (1974, p. 249) used exactly the same words. In this approach, termed by Croizat (1952) "the cult of the petrifact", the age of the earliest known fossil of a group is the age of the group, the location of that fossil is the group's center of origin, and the fossil itself is the group's ancestor.

In contrast, panbiogeography (Croizat, 1964; Craw et al., 1999) has insisted on the distinction between *age of being* and *age of fossilization*, and the idea that fossils are ancestors has been severely criticized by cladists (Gee, 1999; Williams and Ebach, 2004).

Correlating the age of taxa with the age of strata they are endemic to

Discussing the butterflies of East African mountains, de Jong and Congdon (1993) argued that "It seems fashionable to interpret the occurrence of present-day taxa in areas that over 80 Myr BP formed part of Gondwana as an indication of Gondwana origin. However, at least in this case there are good arguments against it. First, there are no [fossil!] indications that the genus *Issoria* existed 80 Myr BP. The oldest butterfly fossils known are about 40 Myr old... [second] it would be very strange for a Gondwana element to be mainly restricted to mountains that are at most 2 Myr old".

Here de Jong and Congdon use two methods of dating: correlating the age of a taxon with the age of its earliest known fossil, and correlating the age of a taxon with the age of strata it is endemic to. Arguments against the first are referred to above. As for the second argument, despite de Jong and Congdon's claim, it would not be strange at all for old taxa to be restricted to much younger strata. Older plants and animals regularly invade younger exposed strata which appear in their vicinity, with the older land surfaces subsequently disappearing—they may be either covered by new sediments or lava flows, or eroded or subducted away (cf. Heads, 1990; Craw et al., 1999). Old taxa often have an ecology favoring sites with youthful soil development (e.g., recently disturbed coastal sands, alpine stream banks). A particular cliff, valley or volcano may be recent, but this does not mean that the habitat *type* is recent. Volcanism often occurs over long periods in the same area (e.g., around plate margins and hot spots) and in theory, the direct ancestors of taxa endemic to Quaternary volcanoes (such as *Issoria* in East Africa, birds of paradise in the New Guinea Highlands, *Lactoris* on Juan Fernandez Islands, and land-snails on Mt Egmont/Taranaki in New Zealand), may have existed on young volcanics less than 2 Myr old, more or less *in situ*, for 80 Myr.

Correlating the age of taxa with the age of paleogeographic events

In a third method of dating, the age of taxa has been correlated with the age of paleogeographic events. This is perhaps the most promising approach but, unfortunately, the applications of this method have, in practice, often been very simplistic. Panbiogeography concluded that earth and life evolve together, not only during continental drift but in general, for example during phases of uplift, subsidence, vulcanism, erosion, juxtaposition by terrane accretion, and many other processes. However, although the early vicariance cladists claimed to be working on a panbiogeographic basis, they adopted a much narrower and rather naïve approach in which it was assumed that any vicariance was the result of continental drift (e.g., Humphries, 1981a,b). The cladists argued that congruence between phylogenies, and between phylogenies and breakup sequences of Gondwana and Laurasia, would be evidence for vicariance caused by breakup. However, much “incongruence” was found and most workers therefore assumed that the distribution patterns could not be the result of vicariance. Because of this they assumed the distributions must be the result of chance dispersal, and this was always assumed to have taken place after continental breakup (e.g., Humphries, 1981a,b).

Panbiogeographers disagreed with this methodology and pointed out (e.g., Croizat, 1981; Craw, 1982, 1983, 1985; Heads, 1985) that the “incongruence” was an artifact, a consequence of faulty analytical method. Phylogenies of Gondwanic groups, even if they responded to breakup by vicariance, would only reflect the breakup sequence if the Gondwanic biota was homogeneous to begin with—a very unlikely supposition. Furthermore, panbiogeographic studies emphasized that the geographic areas used in vicariance–cladistic analysis (such as New Zealand, New Caledonia, New Guinea and Borneo) were being accepted *a priori*. In fact these areas are all geological and biogeographical composites. Using these unanalyzed “areas” as elements in analysis leads automatically to “incongruence”, and the conclusion that patterns have not been caused by vicariance but by later chance dispersal. Detailed analyses of New Zealand (Heads, 1990), New Guinea (Heads, 2001, 2002) and Borneo (Heads, 2003) emphasized that none of these constitute biogeographical regions or centers of endemism (in the strict sense of having any taxa restricted to, and more or less throughout, the area). Instead, the separate component terranes each had independent affinities with other areas.

Despite these attempts to clarify the situation, many authors continue to use present-day areas as units in biogeographical work. For example, in a study of a

Pacific group, *Abrotanella* (Compositae) and allies, Swenson (1995) and Swenson and Bremer (1997) found no simple vicariance among the geographic areas New Zealand, Tasmania, New Guinea and South America. Instead, there was cladistic incongruence, with, for example, Tasmania, sometimes related to New Zealand, and sometimes to Patagonia. Because of this, the authors concluded that the distribution was the result of dispersal following continental drift. However, a panbiogeographic analysis of the actual distribution areas, not the geographic areas, showed clear vicariance. This occurred between *western* Tasmania and *south-western* New Zealand, *eastern* Tasmania and Patagonia; *northern* New Guinea and California/Chile, *southern* New Guinea and New South Wales; *western* Patagonia and Stewart Island, *central* Patagonia and Juan Fernandez, and so on (Heads, 1999). (The “southern”, “northern”, etc., distributions were shown to correlate with geological terranes within the respective areas).

Vicariance–cladistic studies continue to make the same elementary mistake that Swenson and Bremer made. For example, Sanmartín and Ronquist (2004) compared phylogenies of Southern Hemisphere taxa with the breakup sequence of Gondwanaland and found incongruence, at least for plants. They deduced that this could be because the plants are too young to be affected by breakup (the possibility of their being too old, which is very likely, was not mentioned), or because of long-distance dispersal after breakup, which they favored. However, the terminal areas in their cladograms, such as New Zealand, Australia, New Guinea, New Caledonia, are all biogeographically and geologically composite, and the analyses are thus invalid. Sanmartín and Ronquist treated New Zealand, for example, as a single area simply because it was “one unit” at the time of Gondwana breakup, but what is so special about this time? In any case, New Caledonia, which they also accepted as an area, is the result of terrane amalgamation after Gondwana breakup.

Assuming *a priori* that any particular geological event, such as the breakup of Gondwana, is relevant to biogeography is a fatal flaw of much biogeography, both dispersalist and vicariance. In contrast, panbiogeography bases analyses on distributions and only compares these with tectonics after patterns are established. In fact, a great deal of evidence suggests biogeographical patterns involving “New Zealand”, “New Guinea”, “New Caledonia”, etc. were determined by earth history events both prior and subsequent to the breakup of Gondwana.

The three methods for dating evolution have all been used in phylogeography, and their use in calibrating nodes in molecular phylogenies is discussed below.

Phylogeography

Croizat (1977) regarded evolutionary development as “a function of (a) panbiogeography and (b) molecular biology”. The results of molecular systematics produced over the last 15 years represent a great scientific advance. Molecular cladograms are often extremely valuable for biogeographical and general evolutionary studies. The vast amount of vicariance they have revealed is of particular interest and this has led, for example, to a dramatic paradigm shift in marine biogeography (Heads, in press b). In contrast, for reasons discussed below, the dates of nodes inferred in many molecular studies seem almost worthless.

Avice (2000) and others have argued that “Vicariance and dispersal are historical phenomena whose relative roles in particular instances can be weighed on the scales of phylogeographic analysis.” However, the method employed in this phylogenetic judgment involves deriving a date for a divergence event (a node) on a molecular tree from the calibration of this or another node and then comparing this date with an accepted geological model. Thus the calibration is a crucial step and it is shown below that there are major problems with this. Hall (2001) argued that “DNA studies offer one way of determining a time-scale for biological development”, but this is probably one of the few things it doesn’t offer; the time scale is based on calibrations from geology. In addition to major problems with substitution models which are not be examined here, molecular biologists have relied on the age of fossils and other simplistic and dubious means to calibrate their “clocks”. This is a particularly obvious weakness with the phylogeographic approach.

Dating nodes in molecular phylogenies

The idea of a strict, universal evolutionary clock (morphological or molecular) is rapidly falling out of favor with biologists. For example, Rodriguez-Trelles et al. (2004) wrote that “The neutrality theory of molecular evolution predicts that the rate of molecular evolution is constant over time, and thus that there is a molecular clock that can be used for timing evolutionary events. Experimental data have shown that the variance of the rate of evolution is generally larger than expected according to the neutrality theory. This raises the question of how reliable the molecular clock is or, indeed, whether there is a molecular clock. We have carried out an extensive investigation of nine proteins in organisms belonging to the three multicellular kingdoms... We observe that the nine proteins evolve erratically through time and across lineages. The observations are inconsistent with the neutrality theory and also with various subsidiary

hypotheses proposed to account for overdispersion of the molecular clock.”

Likewise, Buckley et al. (2001) admitted that “molecular clocks are notoriously difficult to apply because of many well-recognized problems... the most difficult of which is that different lineages evolve at different rates.”

Norman and Ashley (2000) utilized the extensive fossil record of Perissodactyla to calibrate molecular clocks. From the fossil record they estimated a relatively recent fossil calibration point (divergence of two equid species at 3 Ma) and a relatively ancient calibration point (divergence of Hippomorpha, including equids, and Ceratomorpha, including rhinocerotids and tapiroids, at 50 Ma). Application of these produced greatly different estimates of evolutionary rates and divergence times for both genes they studied. Neither calibration point produced estimates of divergence times consistent with paleontological evidence. The early calibration rate places the separation of the two equid species at greater than 13 Ma, which is “incompatible” with the fossil evidence. (Of course an early age is not truly “incompatible” with the fossil record.) Using the recent calibration rate gives a date that really is incompatible with the fossil record: a separation of Ceratomorpha and Hippomorpha at 8–11 Ma, and Rhinocerotidae and Tapiridae at 6–8 Ma, although fossils of these groups are known before this date. Norman and Ashley criticized the use of molecular clocks, especially when based on single calibration points.

New methods of estimating branch lengths do not assume a strict clock (cf. many contributions in Pennington et al., 2004a; Givnish and Renner, 2004a). However, Near and Sanderson (2004) noted that “With respect to rate heterogeneity, once the model of molecular evolution departs from a simple one-rate molecular clock, the divergence time problem enters a realm of model selection in which the number of models is effectively infinite.”

The idea of a strict molecular clock is less popular now than it was for a few years of the last decade. However, roughly “clock-like” evolution, in which evolution proceeds more or less continuously, although not at exactly the same rate in different lineages, has been a common assumption in biology since the acceptance of evolution. It is seen in the almost ubiquitous idea that greater divergence between taxa generally implies a longer time of evolution. For example, Ehrendorfer and Samuel (2000) wrote that “Judged by their morphological (and molecular) divergence, these [South America/New Zealand] disjunctions, which range from the infraspecific (e.g., in *Hebe*), to the specific (e.g., in *Anemone*), sectional, or even subgeneric level (e.g., in *Fuchsia* or *Nothofagus* p.p.), must be of very different ages...”. Even this rough clock makes the quite unwarranted assumption of more or less equal

rates of evolution in different groups, and authors such as Hutton (1872) have concluded instead that “differentiation of form, even in closely allied species, is evidently a very fallacious guide in judging of lapse of time”. Croizat (1964) also criticized the universal clock approach and made the opposite assumption, namely that different groups in the same region (for example, New Zealand—South America) can and do usually show tremendously different rates, or at least degrees, of differentiation during a single phase of evolution. He also argued that rates can change dramatically within a group and cited potentially great differences between the *time* involved in, and the *age* of, the evolution of a group. He concluded that much evolution has taken place during “phases of modernization” following which there have been long subsequent periods of evolutionary stasis (or only parallel evolution in which genetic distance between groups remains the same). For example, there has been marked stasigenesis in main form of some arachnids for 200 million years (Turk, 1964). The oldest fossil bee (Late Cretaceous) is “astonishingly similar” to living species of *Trigona* (Engel, 2000). McDaniel and Shaw (2003) interpreted a moss species, *Pyrrhobryum mnioides*, which occurs in Australia, New Zealand and South America as a result of Gondwanic vicariance and morphological stasis for over 80 million years. There is also much new evidence in support of rapid, ancient radiations (e.g., Fishbein et al., 2001).

Smith and Peterson (2002) concluded that problems of establishing accurate calibration points “remain formidable”, and in the course of a debate largely concerned with calibration, Conti et al. (2004) described calibration as “one of the most problematic issues in molecular dating analyses”. In their review, Arbogast et al. (2002) admitted that molecular nodes are calibrated by fossils or “particular biogeographical events” (which are often what we are trying to investigate in the first place) and that this can lead to “considerable error”. Surprisingly, the authors devoted only two sentences to this topic which is obviously fundamental to the whole enterprise. This approach—leaving fundamental assumptions unexamined (even when they have been analyzed at length in the 1960s–1980s)—is characteristic of much phylogeography and means that in general the field has become merely a technically advanced form of Wallace/Matthew dispersalism, based on the same concepts and analytical methods.

Whether or not a strict clock is assumed, at least one node on a tree must be calibrated to give the tree a time dimension. Calibrations of nodes in molecular phylogenies have been made using the three techniques cited above that have been used to date evolutionary events in general: calibration with the age of the earliest known fossil, with the age of strata that endemics occur on, and with the age of related paleogeographic events.

Calibrations based on the age of the oldest known fossil

Voelker (1999), like Avise (2000), suggested that “Dispersal- and vicariance-driven speciation can be better tested via the application of molecular clocks [or dated trees in general]” than by congruence of phylogenies and geological split sequences, but admitted that “Although taxa with a good fossil record [but how would this be known?] can be used to calibrate a specific clock [or tree]... the great majority of taxa lack a fossil record suitable for calibrating clocks and thus investigators must use a rate calibrated for other groups [with a fossil record], a problematic... but unavoidable approach.” Of course, while this approach is definitely “problematic”, it is only “unavoidable” in a paradigm which relies on fossils for dating. Bremer (2002) also claimed that “At least some reference fossil is necessary for dating”, and Wikström et al. (2001) wrote that “a single absolute calibration point has to be selected with reference to the fossil record”. These authors ignore a vast panbiogeographic literature and even many orthodox studies which use correlations with paleogeographic events.

In most phylogeographic studies the fossil record is taken at face value and the age of a clade is equated with the age of its oldest known fossil. Node calibrations and the chronology of the whole phylogenetic tree are based on these ages. As indicated above, this is simplistic and misleading.

In a hopeful sign of progress, more and more credence is apparently being given to the principle that the age of fossilization of a group is less, to much less, than the age of being of the group. For example, Smith and Peterson (2002) wrote that “paleontological dates fix only the latest possible time of divergence”. However, while many authors pay lip service to this caveat in their introductions, in their actual analyses most authors overlook it and shift from explicitly citing ages as minima, to citing ages as absolute dates. (The habit of assuming the age of the oldest fossil equals the age of the taxon is evidently deeply entrenched). The authors then assume that older geological events cannot be relevant to the phylogeny, whereas in fact only younger events can be ruled out. In panbiogeography the age of the oldest fossil is never assumed to represent the age of the taxon and so a calibration based on the oldest known fossil cannot be accepted either.

For example, Smitsen et al. (2003) based node calibrations in *Scleranthus* (Caryophyllaceae) on fossil ages and concluded that the genus “diverged within the last 10 million years”, whereas this should have been given as “at or before 10 million years ago”. (This mistake leads inevitably to others: the authors find themselves forced to admit that “Clearly, *Scleranthus* is capable of long-distance dispersal [from Europe to Australasia, direct!] despite lacking any obvious adaptations to facilitate it”.)

Renner et al. (2000) wrote that “Calibration with a pollen-based minimal family age of 90 MY... implied arrivals of *Laurelia novae-zelandiae* in New Zealand at ~33 MYA and arrival of *Nemuaron* in New Caledonia at ~25 MYA” (italics added). However, these dates for “arrival” (assuming a dispersal model) will also in fact be minimal ages.

Renner et al. (2001) claimed that they “estimate absolute ages for key divergence events in the history of Melastomataceae from substitution rates... calibrated with different fossils”. Again, these estimates are minimum, not absolute, dates. Nevertheless, based on these dates the authors deduced the usual northern center of origin followed by Neogene long-distance dispersal into South America, Africa and Asia.

In their study of Melastomataceae tribe Melastomeae, Renner and Meyer (2001) wrote that their assignment of the oldest known fossils of the tribe to the basal node in a tree could result in “potential underestimation” of the tribe’s age, as the fossils “may represent a latter stage during the clade’s history”. This is very likely to be true. They also argued that the Eocene age of the earliest fossils of the family “places an upper [i.e., oldest] boundary on the age of Melastomeae”, but this is not logical. This Eocene age is a minimum age; it is not a maximum age for either the family or any group in it. Renner and Meyer used these arguments and the fact that all known fossils are in Eurasia to support a center of origin in western China, followed by long-distance dispersal around the globe.

Following their calibration of a clock for *Myosotis* (Boraginaceae) based on the age of the earliest known fossils from New Zealand, Winkworth et al. (2002) suggested that the “earliest possible Southern Hemisphere ancestor existed 2.0–14.7 MYA”, whereas this is in fact the latest possible date.

Wagstaff et al. (2002) calibrated a clock for the South Pacific *Hebe* complex (Scrophulariaceae) based on mid-Miocene fossils (15 Ma), and indicated “divergence estimates” of the “Australasian species clade” at 9.9 Ma and the “*Hebe* clade” at 3.9 Ma. Again, these should have been referred to as minimum estimates. They admit the possibility that older fossils may be found, but suggest that “it would be inconsistent with this [fossil] record to assume that divergence... occurred in Gondwanan (Cretaceous) or earlier times”. However, inferring older events as causative would not be “inconsistent” with the record, only invoking younger events would be.

Perrie et al. (2003) calibrated a clock for *Polystichum* (Dryopteridaceae) based on the first known fossils of the family at 140 Ma. (They wrote that the fossil record of ferns is “particularly good”, but no evidence for this was cited.) From this they deduce that “It might therefore be assumed” that *Polystichum* and *Dryopteris* have differentiated “within the last 140 Myr”, but this is not

correct. The family is older than 140 Myr, and so the split between these two genera may well be too.

Hurr et al. (1999) calibrated a clock for *Sophora* (Leguminosae) using the oldest known fossil of *S. tomentosa* from 30 Ma (*S. japonica* has older fossils). They inferred that this “yields times of origin for *Sophora* sect. *Edwardsia* between 9.6 and 8.9 million years ago” but, again, these are minimum ages and a correct statement would read: “at least 9.6–8.9 million years ago”. Their claims that they can therefore “eliminate vicariance”, that long-distance dispersal “must” account for the distribution, and that their results give “clear indication of recent origin” and “demonstration of long range dispersal” are thus false. They write that “it is possible that the fossil data underestimates the time of origin of the genus in New Zealand”, but fossil data *always* underestimate times (Benton and Ayala, 2003; Conti et al., 2004).

For the tenrecid insectivores of Madagascar and Africa, Douady et al. (2002) wrote that the oldest fossils are Early Miocene (c. 20 Ma), and that the separation of Madagascar from mainland Africa occurred between 120 and 165 Ma. They regarded the latter date as “well before the origin of tenrecids”, but this is not a logically valid conclusion. In fact, their studies gave a date for the divergence of the tenrec subfamilies at 53 Ma, which they claimed is “much older than the fossil record would suggest”. In fact this date is compatible with the fossil record which only gives minimum ages.

Two New World plant families, Bromeliaceae and Rapateaceae, each have a single African species. Givnish et al. (2004) calibrated a molecular clock for the families using “Cretaceous fossil information to place minimum ages on eight monocot nodes”. Despite this, Givnish and Renner (2004a) found that the African species are the result of divergence “no earlier than the past 7–12 million years”. This estimate, given as a maximum age (and used to deduce trans-Atlantic long-distance dispersal), is based on a calibration which gives a minimum age.

Heenan et al. (2002) calibrated a molecular clock for New Zealand Cruciferae based on results in Koch et al. (2000) which, although Heenan et al. did not mention it, are based on oldest known fossils of the family. Heenan et al. then estimated the “period of evolution” of *Ischnocarpus novae-zelandiae* at “equivalent to about 1 million years” and *Cheesmania latisiliqua* at “2.1–3.5 million years”, whereas, again, these are minimum ages, not “periods of evolution”. Koch et al. (2000) wrote: “Pollen from close relatives of *Cardamine* and *Barbarea* [Cruciferae] is common in geological samples from the Pliocene (2.5–5 Ma). Therefore, we assume that *Cardamine* and *Barbarea* diverged about 6.0 MYA”. No reason for this assumption is given. Why accept only 1 Myr difference between oldest fossil and age of the group? Why not 2, 10 or 20 Myr? In fact Koch et al.

(2000) were more open than Heenan et al. (2002) about the problems their methods faced and wrote that “Estimates of divergence times are problematic” and also referred to the “great uncertainty associated with dating speciation events in the fossil record.”

Because of these and other assumptions, the method of dating nodes using oldest known fossil can hardly constitute a strong test of vicariance versus dispersal, or indeed anything else.

As Conti et al. (2004) pointed out, “When [oldest] fossils are used to provide minimal ages of the subtending stem lineages, and if those minimal ages are interpreted as estimates of actual ages, then one inevitably obtains a systematic underestimation of divergence times.”

Some more critical assessment of calibration has recently begun to appear. For example, in a paper on molecular dating by Van Tuinen and Hadly (2004), “the focus is primarily on fossil calibration error because this error is least well understood and nearly universally disregarded.” However, these authors concentrated on error in dating strata and error in placing fossils on a tree, and did not deal with sampling error in the fossil record, which is likely to be an even greater source of error (witness the new oldest fossils cited above).

Magallón (2004) noted that “a temporal difference of unknown magnitude exists between lineage splitting and a clade’s appearance in the fossil record... Absence of a fossil species at a given level below (or above) its observed stratigraphic range means either that the species had not originated (or was extinct) by the time of deposition or that the species was alive but was not preserved in the fossil record.” In fact, fossils may have been preserved, they just haven’t been found yet. Magallón has overlooked the importance of the huge sampling error in fossil collection, highlighted by the “new oldest” fossils cited above. A thick stratum, representing a very long period of geological time and large geographic space, may be represented by a single, small fossil locality (or none). A geologist can only crack open so many rocks in a day and may only have limited time at a site.

Magallón (2004) discussed various methods proposed for calculating confidence intervals for estimated dates for clades from the fossil record; these are hardly convincing and Givnish and Renner (2004a) noted, rather unenthusiastically, that “The construction of stratigraphic confidence intervals from the temporal distribution and abundance of known fossils *may, to a limited extent*, help compensate for inherent uncertainties in the record” (italics added). As Brochu (2004) pointed out, methods for assessing the confidence limits of a fossil range or extracting an actual divergence date from the fossil record “require information about stratigraphic sampling that we simply do not have for most groups... and these

methods can themselves be very sensitive to a priori assumptions.”

Calibrations based on the age of the strata to which taxa are endemic

A common error that biologists make in reading geology involves taking geologists’ suggestion that “there is no evidence for land” in a region for a particular period to mean “there is evidence for no land”. This has led biogeographers to believe that the age of an island (i.e., the currently exposed strata) “indirectly places a maximum age limit on any endemic plants that have evolved *in situ*” (Baldwin et al., 1998). Likewise, Richardson et al. (2001b) claimed that the age of endemic taxa on volcanic islands can be “precisely measured” by dating the age of the volcanic strata they currently live on. This is simply not true, as it assumes the species have always lived, and only ever lived, on that particular volcanic stratum. These authors dated the “radiation” of the South African Cape flora from the fact that some species of the shrub *Phyllica* (Rhamnaceae) and its sister genus *Nesiota* are endemic to volcanic islands of known age. They wrote that “two critical points of calibration were known”. First, one species of *Phyllica* occurs only on Mauritius and Réunion, and as the latter is only 2 Myr old, they assumed the species dispersed there from Mauritius, the older island. They acknowledged no other possibility, such as the former presence of other islands close to or at the current location of Réunion. Secondly, *Nesiota* is endemic to St Helena, known to be 14.3 Myr old, and so it was inferred that this was the age of the split between *Nesiota* and *Phyllica*.

After landbridges were discredited, biologists assumed that this left long distance dispersal from a mainland or from other current islands as the only way taxa on “oceanic” islands could establish. This overlooks the fact that volcanic islands are individually ephemeral but are always forming at oceanic plate margins (such as St Helena) or at hot spots (such as Mauritius and Réunion). It is unlikely that the current volcanic islands at these sites are the first to be formed there; probably they are only the latest in a long history of island formation and disappearance.

The possibility that plants on volcanic islands might be substantially older than the very young stratigraphy they currently grow on, for example by surviving on older strata or islands that are now eroded, buried or subsided, negates the central argument of dating taxa with age of strata. Lactoridaceae are endemic to the geologically very young Juan Fernandez Islands. The family has been dated to more than 125 million years using a molecular clock (Wikström et al., 2001), so some authors might argue it is obvious that they were somewhere else before the current islands emerged.

However, *Lactoris* may have always been in this region, surviving on small, geologically ephemeral volcanoes. Many other authors have also recognized this phenomenon.

For example, unlike many botanists who have denied that there is a true Polynesian flora, Philipson (1970) argued that plants endemic there, such as *Meryta* (Araliaceae), *Tetraplasandra* (Araliaceae), *Fitchia* (Compositae), *Sclerotheca* (Campanulaceae), *Vaccinium* sect. *Macropelma* (Ericaceae) and many others, indicate that “the southern Pacific islands must be credited with a flora specific to this region... Clearly land has been present for long periods in this area of the Pacific because well-marked genera are endemic to it. The flora characteristic of this region could survive provided a few oceanic volcanoes projected above the sea at all times. Such oceanic islands characteristically rise and fall relative to sea-level so that they are precarious footholds for a flora, but collectively they form a secure base.” In this view, new individual islands will be colonized by ordinary, everyday movement, an observable ecological phenomenon (very different from long-distance dispersal and founder effect) which functions using ordinary means of survival.

Cowie (1996) reached similar conclusions in his discussion of the families of large Pacific island land-snails, Partulidae, Amastridae and Achatinellidae: “dispersal from island to island as new islands form and old islands disappear seems the only logical explanation for the continued presence of these groups [which are] generally considered relictual in the Pacific.”

Paleogeographic details of islands are generally not known and probably never will be known. Small islands and hotspots come and go, and geologists have little expertise or interest in knowing whether any land, even a small area, remained above water at a particular time. Volcanoes are geologically ephemeral features, often lasting no more than a million years, and usually only belts or phases of volcanism, not individual volcanoes, show up in the record. On the other hand, living taxa, at least in certain groups, are capable of surviving as a metapopulation more or less *in situ* on very small areas of land coming and going in a sea. Although the distribution patterns of such taxa are hardly ever taken seriously because of the assumption of chance dispersal, and so not studied in detail, they probably provide much more information on paleogeography than the dates of currently exposed volcanic strata.

The sort of argument used by Richardson et al. (2001b)—that an endemic on a volcanic island can only be as old as the island or have reached it from other current land masses by long-distance dispersal—is a fundamental tenet of Matthewian biogeography, but is based on unwarranted assumptions. Nevertheless, their article was published in a prestigious journal, and the method they used and the “rates of evolution” they

deduced will be cited by dozens of other studies as authoritative. Already, for example, Kimball et al. (2003) have dated the core group of *Coreocarpus* (Compositae) as one million years old “because the basal species is endemic to a volcanic island that emerged in the past million years”, and these authors cited Richardson et al. (2001b) as support for this methodology.

Calibrations based on the age of paleogeographic events

Most of the standard correlations between distribution and paleogeographic events used to calibrate clocks are highly simplistic. A classic example is the final rise of the Isthmus of Panama at about 3.5 Ma. This is one of the most important dates in dispersal biogeography as it is supposed to mark the beginning of the “Great American Interchange” of terrestrial faunas, one of the central pillars of the dispersalist approach (Wallace, 1876; Stehli and Webb, 1985). The rise of the Isthmus is also widely assumed to have brought about vicariance between Atlantic and Pacific marine taxa and so the 3.5 Ma age of the isthmus is very often used as the basis for clock calibrations. In other words, bad biogeography is used to produce bad calibrations which are then used to produce more bad biogeography. However, many authors have pointed out (so far to little avail) that many Atlantic/Pacific pairs of taxa must have diverged well before the final rise of the isthmus (Heads, in press b). Croizat (1975) questioned the significance of the Isthmus of Panama and emphasized instead the complex geological history of the Colombia-Central America region. Other geologists and biologists have also suggested earlier isthmian links between North and South America in the late Mesozoic and early Cenozoic that may have led to Pacific/Atlantic isolation and differentiation of marine taxa.

White (1986) suggested this for nearshore fishes (Atherinidae) and Savin and Douglas (1985) also referred to many openings and closings of the Isthmus. Rosen (1988) observed that although the differentiation of the main centers of endemism for reef corals, the Atlantic and Indo-Pacific regions, was often attributed to the Pliocene emergence of the isthmus, the emergence actually long postdates faunal differentiation of reef corals (early Cenozoic based on age of fossilization, and so possibly much older).

de Weerd (1990) commented that “The Pliocene uplift of the Panamanian Isthmus is generally recognized as the vicariance event leading to sister-group relationships at both sides of the Isthmus... The conspecificity of the Brazilian and eastern Caribbean populations of *Millepora squarrosa* [a hydrocoral] does not fit very well with this timing, since the age of the Amazon-Orinoco barrier has been hypothesized... to be of Miocene age, thus older than the Isthmus. Glynn

(1972, 1982) has presented the hypothesis that a restriction of flow across Central America occurred already before the rise of the Isthmus... It is suggested that the speciation of the ancestor of *M. squarrosa* and [its Indo-Pacific sister species] *M. platyphylla* into these species has taken place before the rise of the Isthmus..."

Likewise Knowlton (1993) suggested that at least some of the trans-Panama species pairs of the snapping shrimp *Alpheus* may have diverged before the final closure of the Panama seaway, in a pattern of staggered rather than simultaneous isolation. Knowlton and Weigt (1998) estimated times of separation of trans-Panama pairs of *Alpheus* ranging from 3 to 18 Ma for 15 species pairs. They assumed that the divergence of the pair with the least difference was due to Isthmus closure, but this pair too may have diverged well before this.

For the *Strombina* group (Scolumbellidae) of gastropods in the Caribbean and Eastern Pacific, sequential vicariance was also proposed based on fossil evidence of divergence prior to 3.5 Ma; there was already substantial divergence at the subgeneric level at 5.0 Ma (Jackson et al., 1993). Banford et al. (1999) suggested that divergence between Pacific and Atlantic members of Spanish mackerels (*Scomberomorus*: Scombridae) took place at around 6.3 Ma, again predating the closure of the isthmus.

For trans-isthmian species of the muricid *Plicopurpura*, molecular data give a predicted time of divergence of between 5.6 and 11.4 Ma, "consistent with the hypothesis that many geminate species [closely related species pairs] were divided long before the final closure of the Panama seaway" (Cunningham and Collins, 1994). These authors concluded that "Paleontological and molecular data agree that the separation of taxa on either side of the Isthmus of Panama was not a singular event, as had previously been supposed, but most likely took place over millions of years".

These conclusions have been almost universally ignored by the many authors who continue to rely on the final closing of the Panama seaway to date nodes on trees and calibrate molecular clocks. As Knowlton and Weigt (1998) concluded: "Many past studies may have overestimated rates of molecular evolution because they sampled [trans-Panama] pairs that were separated well before final closure of the Isthmus."

Finally, Muss et al. (2001) suggested that because the Atlantic and Pacific species of *Ophioblennius* probably diverged prior to the closure of the Isthmus of Panama, "a geologically calibrated clock is unavailable". This indicates the narrow approach of many contemporary studies and the excessive reliance placed on this single feature. Of course there are many other geological features in the Pacific and Atlantic Oceans that could be used in calibrations, but for some reason never are.

Another example of calibration based on naïve correlations with tectonics is provided by Buckley et al.

(2001) in a study of New Zealand cicadas. The authors based their calibration on an inferred date of 5 Ma for the radiation of the "alpine *Maoricicada* species", "because the New Zealand alpine habitat is only 5 million years old". They probably read that the *Southern Alps* are only 5 Myr old, but there is a vast amount of evidence for mountains in New Zealand prior to this (Heads, 1990). In any case, by using this calibration to estimate the ages of *Maoricicada campbelli* haplotypes, Buckley et al. ignored the fact that *M. campbelli* does not require alpine habitat; it occurs from "low elevation to subalpine regions". Indeed, most of the "alpine" species of *Maoricicada* occur below alpine altitudes (10 out of 14 have populations at less than 1000 m, and several are in the lowlands; Dugdale and Fleming, 1978).

Case studies in phylogeography

Studies on echinoderms

Waters and Roy (2004) sought to explain the distribution of the starfish *Patiriella exigua* which ranges from St Helena to SE Australia and Lord Howe Island. They claimed that this standard Indian Ocean-centered distribution is "unusual", and because their cladogram for the populations is more complex than a simple Africa/Australia split by continental drift they inferred, incorrectly, that the distribution cannot be the result of vicariance. This is an example of assuming that vicariance can only be the result of Gondwana breakup (cf. Swenson and Bremer, 1997; Sanmartín and Ronquist, 2004; discussed above). The distribution of *Patiriella* may involve vicariance prior to the Africa-Australia split and/or the relevant geology may be more complex than a simple split. In fact, the genetic clades within the species show striking vicariance—Waters and Roy term it "marked phylogeographic structure"—"evident across small geographic scales in Australia and South Africa", which basically means throughout the species' range. As the authors admit, this structure "indicates that gene flow among populations may be generally insufficient to prevent the local evolution of monophyly." In other words, dispersal is not important and vicariance is. In fact, the basal split in the species is between the Cape Town-Amsterdam Island populations and all the others. No explanation for this or any of the other vicariance is given; Waters and Roy merely state that "Organisms that possess strong migratory ability can undergo major range expansions through the colonization of new regions." However, there is an important difference between what an organism can in theory do in terms of colonization and what it actually does in practice. In fact, any organism, given suitable ecology, can undergo massive range expansion, whether

or not it has especially ‘strong migratory ability’, since all organisms have the power to move at some stage in their life cycle. For example, there are weedy species of snails, worms, etc. that have invaded very large areas. Conversely, many species with very high ‘migratory ability’, such as albatrosses and fruit bats, can in theory, but do not in practice, undergo major, rapid range expansion. In any case, strong migratory ability in *Patiriella* does not explain the main pattern of ‘marked phylogeographic structure’.

Despite their interesting evidence of vicariance, Waters and Roy concluded lamely that ‘‘small’’ genetic divergences between African and Australian haplotypes ‘‘strongly suggest Pleistocene dispersal’’. As pointed out by many authors, degree of divergence is a guide neither to the time involved in evolution, nor the age of that evolutionary event. Waters and Roy claim that their study ‘‘is one of the first to provide convincing evidence’’ and even ‘‘compelling evidence’’ of long-distance rafting. But examples of very closely related taxa and populations from distant localities—which, along with highly structured vicariance, is all they have demonstrated—have been known for centuries. The interpretation of these patterns remains controversial.

Waters and Roy (2004) argued that ‘‘Phylogeography has transformed biogeographical research, a field previously dogged by rhetoric and speculation, into a rigorous discipline centered on hypothesis testing (Wallis and Trewick, 2001).’’ This is simply propaganda and in fact, as Ebach and Humphries (2002), Nelson (2004) and others have argued, there has been no transformation at a conceptual level, but rather a notable regression to the theory of the 1940s. The phylogeographers have confused technical progress with conceptual advances. The theory and practice of phylogeography are not centered on hypothesis testing but on reiterating the preconceptions of Matthew (1915) and the New York school of zoogeography. Its practitioners are unaware of this because they themselves are often not biogeographers and have little knowledge of that subject or its history.

Waters and Roy (2004) concluded their paper with the revealing sentence: ‘‘Several recent phylogeographic studies have included statistical tests of dispersal hypotheses and have been published in high-profile biology journals’’ (references cited), as though the ‘‘profile’’ of the journal should be used in assessing whether or not a study’s conclusions make sense.

In a study of the widespread sea-star genus *Coscina-asterias*, Waters and Roy (2003) reached similar conclusions to those of their 2004 paper. Again, haplotypes exhibited ‘strong phylogeographic structure’, and again any mention that this is vicariance is carefully avoided. The curious claim is made that the presence of ‘related haplotypes’ on Tasmania and New Zealand ‘suggests that long distance dispersal is

an important biogeographical process’’, but this relationship could also be caused by vicariance. The assumption that degree of difference is proportional to time and that the calibration is reliable is made throughout the paper. Thus, what are supposedly ‘shallow’ genetic differences mean that vicariance can be ‘clearly’ rejected, and again the authors boast that their data provide ‘strong’ evidence that the distribution is attributable to long-distance dispersal. Waters and Roy write, correctly, that while a number of studies use the final rise of the Isthmus of Panama as a means of calibrating molecular clocks, ‘such clock calibrations should be treated with caution’. However, they themselves apply molecular calibrations derived from sister taxa isolated by the Isthmus of Panama. This is the basis of their ‘strong’ evidence and ‘clear’ rejection of vicariance.

Studies on Madagascar

1. Chameleons

Several studies (Klaver and Böhme, 1986, 1997; Hofman et al., 1991) have supported a vicariance model for chameleon biogeography. A recent phylogeographic analysis of 52 chameleons (Raxworthy et al., 2002) found six major clades, with the terminal three distributed in Madagascar-Seychelles, Africa-India, and Madagascar-Comoros-Réunion. This very interesting overall vicariance is not even mentioned by the authors. Instead, they claim that the cladogram cannot be reconciled with ‘proposed’ Gondwana breakup models. However, again, the chameleons may be older than the breakup, or the relevant geology may be more complex than a simple split. Neither of these possibilities is mentioned by Raxworthy et al. who instead invoke an ‘out-of-Madagascar’ model. This is a classic case of assuming over-water, chance dispersal, rather than actually analyzing patterns and investigating whether, for example, the two clades that occur in Madagascar are distributed differently there. Raxworthy et al. found what they called ‘‘corroborating evidence’’ for oceanic dispersal in the fact that the volcanic Comoro Islands, formed between 0.13 and 5.4 Myr ago, have endemic chameleons, whose direct ancestor ‘‘could only’’ have reached the archipelago by means of ‘‘oceanic dispersal’’. This makes the drastic assumption (again, not mentioned) that there has been no prior land in the area. (McCall, 1997, has suggested emergent land through Eocene-Miocene time along the Davie Fracture Zone of the Mozambique Channel, and the Comoros have formed at a hot spot; Kearey and Vine, 1996.) Raxworthy et al. proposed that molecular clock divergences, based on estimated rates for other ectotherm vertebrates, are also ‘consistent’ with the post-Gondwana model, but they do not say how these

ectotherm rates were calibrated. Rieppel (2002) asserted that Raxworthy et al.'s study "confirms that dispersal is important". However, he also noted that the occurrence of groups such as chameleons, freshwater fishes and terrestrial mammals in Madagascar "has been seen as paradoxical", since their origin is assumed to postdate the separation of Madagascar and because they are considered to be poor dispersers. He concluded by admitting that "How chameleons managed to disperse across the ocean must remain a matter of speculation" (cf. Waters and Roy's, 2004 claim, cited above, that phylogeography has moved beyond speculation). Thus phylogeography, like Matthewian dispersalism in general, does not solve biogeographical problems but simply leads into a morass of mysteries and paradoxes.

Townsend and Larson (2002) studied 57 chameleons and produced a rather different cladogram to that of Raxworthy et al. They found a level of divergence about 10% lower than expected for a cladogenesis associated with Gondwanic fragmentation, but did not indicate how their clock was calibrated.

2. Tree frogs

In hyperoliid tree-frogs, the Madagascan genus *Heterixalus* is sister to *Tachycnemis* of the Seychelles, and this grouping has usually been explained by continental drift (Richards and Moore, 1996) and vicariance. However, Vences et al. (2003) argued that "Although vicariance often offers more appealing explanations, dispersal hypotheses should not be disregarded for Malagasy frog radiations... [they cite "island hopping from Africa" and "rafting"]... Presence of the Hyperoliinae [tree frogs] on the Madagascar-India continent implies a very early age of their evolution [and vicariance]. Such an assumption, however, meets with several contradicting facts. The first problem is the absence of these frogs in South America... Madagascar was apparently connected with South America via the Kerguelen plateau and Antarctica in the late Cretaceous... It is difficult to understand why such a vagile group would not have been able to colonize [South America]." The absence of these Madagascar-Seychelles frogs from South America is not a "contradictory fact", simply a standard pattern. Vences et al.'s argument does not follow logically but is a classic dispersalist line of reasoning. It was used by Wallace (1998), for example, in arguing that Madagascar cannot have been connected with southern Asia at the time that groups such as squirrels, deer and antelope existed in Asia, otherwise they would have migrated into Madagascar. Likewise, Vences et al. also observed that the Madagascar-Seychelles tree-frogs were not in India either, but again, why should a group on the Madagascar-Seychelles-India continent be assumed to have ever ranged

throughout? In another example, authors often seem to feel that if a group was in part of Gondwana it must have been throughout, and if it wasn't throughout, then it can't have been in Gondwana at all. In other words they assume that the Gondwana biota was homogeneous, which is surely unreasonable. None of the current continents (except perhaps the glaciated wastelands of the north) have anything like a homogeneous biota, and a supercontinent would probably be even more diverse. Just because a "vagile" group *can* colonize, does not mean that it actually *will*. Practically any group on a continent shows vicariance within the continent. A classic insular example of lack of dispersal are the birds of paradise on New Guinea, which should be able to colonize the large islands of the nearby Bismarck Archipelago easily but for some reason never have (Croizat, 1958; Heads, 2001, 2002).

Vences et al. (2003) also suggested that the "low" genetic distance between the Malagasy and Seychelles frogs imply that they "may not well be explained by vicariance", which would require "unprecedentedly low substitution rates". However, they admitted that "molecular clock estimates are often of limited value... and no calibrations are so far available for the Hyperoliinae." In a different approach, Bossuyt and Milinkovitch (2001) calibrated the Madagascar versus India-Seychelles split in Ranidae (i.e., Mantellinae versus Rhacophorinae) using the date of the geological split between these areas (88 Ma).

3. Tenrecs

Tenrecs (Insectivora, Tenrecidae) comprise four sub-families, one on mainland Africa and three endemic to Madagascar. Using a molecular clock, Douady et al. (2002) estimated the split between the African subfamily and one of the Madagascar subfamilies at 53–51 Ma, much older than the oldest fossil tenrecid (20 Ma). No information on mode of calibration of the tree or even citation of relevant literature was given. The node was obviously not calibrated at the date of the split of Africa and Madagascar (165–120 Ma), although this would be reasonable (cf. Bossuyt and Milinkovitch, 2001; cited above). Based on their calibration, Douady et al. argued that "dispersal events" are required and concluded, as usual, that "the mechanism by which tenrecids arrived in Madagascar remains unclear". These burrowing animals would seem to be unlikely candidates for long distance dispersal by rafting or island-hopping.

Discussion

The most critical point in inferring dates from a molecular phylogeny is how the initial dates were calibrated. In many cases this is simply not mentioned

(e.g., Lockhart et al., 2001; Douady et al., 2002). In most cases (for example, Richardson et al., 2001a; Pennington et al., 2001; Verboom et al., 2003), calibration is based on results from another paper, which is cited, but no other information on calibration is given. Verboom et al., 2003, cited Bremer, 2002, who in fact used age of oldest known fossils). The chronology in a paper by Klak et al. (2004) is crucial to their whole argument of “unmatched tempo”, “remarkable speciation bursts”, “very recent and very rapid diversification”, etc. However, it is based entirely on calibrations given in Wikström et al. (2001) and no other information is given as to how the calibration was made. This makes it impossible to assess the value of the results in Klak et al.’s paper without referring to the Wikström et al. study (which was in fact based on the age of oldest fossils). This practice of not giving details on the mode of calibration is unsatisfactory and already involves a huge literature that is difficult to assess. It should at least be mentioned whether the original estimate is based on correlation with fossil age, with the age of strata, or with the age of paleogeographic events. The trend towards not citing mode of calibration is more than just a minor technical oversight; it reflects the attitude that the dates inferred in any molecular study are more or less correct and of great interest, and that the method of calibration is of only minor relevance.

Other methodological inconsistencies in calibrating molecular trees have been pointed out by some critics. For example, Shaul and Graur (2002) noted that the choice of calibration points for converting genetic distance to evolutionary rate is high on the list of factors contributing to variation in divergence date estimates for the same event. They investigated the use of secondary, or indirect, calibration points—divergence time estimates derived from one molecular dataset on the basis of a primary external calibration point (usually age of the oldest fossil) which are used again independently of the original external calibration point on a second dataset. Secondary time estimates are used frequently. “Unless particular care is exercised, this practice leads to internal inconsistencies, and the inferred dates of divergence are by necessity unreliable.” “Derivation of divergence dates from molecular data is a complicated proposition even at the best of times... and using secondary calibration times complicates matters unnecessarily. As an extreme measure we would suggest not to derive divergence dates from molecular data at all.”

Graur and Martin (2004) extended these observations in an amusing critique (but see van Tuinen and Hadly, 2004). They noted that molecular evolutionists (mainly Hedges and colleagues) have produced a plethora of seemingly precise dates for a range of divergence events. The appearance of accuracy has an “irresistible allure”, but the “illusion of precision” was achieved mainly

through the “conversion of statistical estimates into errorless numbers”. By employing such techniques successively, time estimates were made to look deceptively precise. They relate “a dating saga of ballooning inapplicability and snowballing error” in which molecular equivalents of Archbishop Ussher’s date of the first day of creation (23 October 4004 BC) “have been mass produced in the most prestigious biological journals... As the saga proceeds, fewer and fewer estimates are derived from the original synapsid-diapsid calibration event and—subtly but surely—more and more are based on the secondary, tertiary and higher order derivations... The continuation of the saga is as predictable as it is outlandish... We might ultimately be able to tell whether the human–chimpanzee divergence occurred on a Monday or not... The appearance of accuracy and the high-quality artwork have resulted in hundreds of citations in which such dates were accepted as factual. Unfortunately, no matter how great our thirst for glimpses of the past might be, mirages contain no water... Despite their allure, we must sadly conclude that all divergence estimates discussed here... are without merit.”

Apart from the obvious technical advances, molecular biogeography, or phylogeography, resembles traditional biogeography in many ways, and both study the spatial distributions of characters and taxa. The results of the former are notable for the very high levels of vicariant phylogeographic structure they have revealed. Perhaps the main way in which phylogeography differs from previous biogeography lies in its claim to be able to date evolutionary events. Because of this, phylogeography also claims to be able to test whether an event is due to vicariance or dispersal. However, there are notable difficulties with this line of reasoning. Perhaps the most common fallacy involves calibrating nodes on a tree with the oldest known fossil of a group. The nodes are then given as absolute or maximum dates, and older geological events are dismissed as irrelevant. However, in fact the nodes will all indicate minimum dates, and only younger geological events can be disregarded. Problems with substitution models are much more difficult and probably more serious, but are not addressed here.

It does appear that modern phylogenetics and phylogeography have reverted to an early 1960s, pre-Brundin outlook. A good example of this is the erroneous idea often used in phylogeographic work (but criticized by Sparks, 2004), that small sister taxa—“basal” groups—are primitive and ancestral, and located near the group’s original center of origin. In fact, a “basal” group is just the smaller of two sister groups. Both will have the same age and neither one is derived from the other.

Thus, phylogeography generally presents a technically advanced form of founder dispersalism, based on the same key concepts of “center of origin” and “means of

dispersal". The modern phylogeographers are the epigones of the early twentieth century dispersalists, held in thrall to the idea of dispersal from a point center of origin, the simplicity of which "captivates the mind" (Darwin, 1971). Like all the German idealists they accept that the ancestor of a group was originally monomorphic and that the descendants have diverged (not evolved in parallel) and, in particular, they share with Mayr (1942) and Hennig (1966) the idea that the most primitive, "basal" taxa occur at the center of origin, while the advanced ones have migrated away (the "Progression Rule"; cf. Avise, 2000).

In sum, as Ebach and Humphries (2002) noted: "Phylogeography has re-invented dispersal biogeography... Phylogeography is limited in its perspective, as it has not overcome the logical hurdles already addressed in cladistic biogeographical methodology over the last two decades." Humphries (2000) observed that "despite four decades of analytical criticism, narrative biogeography [based on Hennig's "progression rule" or its opposite] still seems to persist and is indeed growing with renewed vigour amongst contemporary evolutionists. Fossils and ancestors still assume cardinal importance and centers of origin are alive and kicking." Nelson (2004) agreed, writing that the paleontology of earlier authors, especially Matthew and Simpson, "is revived in molecular systematics of the present, in the search for ancestors and centers of origin."

Cladistics was an attempt to get away from using an absolute degree of difference in systematics. But in modern phylogeography the degree of difference is still taken to be meaningful and even of fundamental importance, especially with respect to time. This again follows the ideas of Matthew (1915) who wrote, for example, that "the Malagasy mammals point to a number of colonizations of the island by single species of animals at different times." Degree of divergence, whether molecular or morphological, may have very little to do with the age of a group, and may indeed be determined largely by the prior evolutionary potential of the group. Under this view, in any given phase of evolution, for example, during a period of rifting, some groups will diverge to generic level, others to species level, and others may diverge only cryptically or not at all. This explains why the same biogeographical pattern is always held by taxa of different rank (Heads, 2004). The different groups sharing the same distribution always involve differing degrees of differentiation due to differing original genetic potential and thus different responses in a single phase of evolution. Phylogeography, however, assumes that the pattern, and the differing degrees of difference, are due to different phases of evolution and thus sees the groups as having different histories. The biogeographical pattern is assumed to be a pseudo-pattern caused by chance congruence. This is just chance dispersal all over again.

In exemplary molecular studies of cichlid fishes, Sparks (2004) and Sparks and Smith (in press) found two main clades in the family, one in Madagascar, Africa and America, and one in Madagascar, India and Sri Lanka. They concluded that these relationships "are congruent with prevailing hypotheses regarding the sequence of Gondwanan fragmentation and a vicariance scenario to explain the current distribution of cichlid fishes."

Sparks observed that "A vicariance scenario for cichlids is disputed by some paleontologists, given that acanthomorphs do not appear in the fossil record until the Upper Cretaceous..., that there are no unquestionable Cretaceous perciforms... and that representatives of Madagascar's extant freshwater fish groups are absent from Cretaceous deposits examined to date on the island... However, there is a global paucity of early Cretaceous through Mid-Paleocene freshwater teleost fossils which is presumably attributable to a scarcity of fossil-bearing freshwater rocks of Cretaceous age... Given that there is a notable gap in the acanthomorph fossil record extending from the Late Cretaceous to the Late Paleocene era, by which time an incredibly diverse fauna has evolved... there is no reason to dismiss the possibility that the fossil record is likewise misleading to date with respect to the origin of percomorph fishes..." Recent discoveries of well-preserved fossil cichlids from the Eocene of Tanzania "illustrate just how quickly and substantially our notions have changed regarding a time of origin for cichlid fishes". These fossils are dated at 46 Ma, whereas prior to their discovery and accurate dating cichlids were only known back to 36 Ma. The fossil taxa "appear to be very derived and similar to modern African lineages".

"Rapid diversification of cichlid lineages (especially east African forms) has been reported time and again in the literature; however, here we have a well-documented example of morphologically conserved forms persisting in eastern Africa for nearly 50 Myr. What do these recent fossil discoveries imply about the age of cichlid fishes? If 46 Myr fossils appear virtually indistinguishable from modern forms, and are nested well within the African assemblage, certainly cichlids are much older even than these fossils (i.e., Cretaceous in age). Moreover, topologies recovered for Cichlidae and aplocheiloid killifish, the only two groups of freshwater fishes in Madagascar with broad Gondwanan distributions, are not only congruent with each other, but also with prevailing hypotheses regarding the sequence of Gondwanan break-up in the Mesozoic. These repeated patterns are intriguing and may well point to a common cause."

Note that Sparks and Smith did not present a calibrated molecular clock or tree with precise dates—this would have added very little to their clear and logical conclusions. Their emphasis is instead on the

topology of the tree, the distribution of the clades, and a critical reading of the available fossil record.

Conclusions

In a special issue on intercontinental tropical disjunctions (Givnish and Renner, 2004a), Givnish and Renner (2004b) noted that all the contributions calibrated molecular trees using the age of the oldest known fossil of the group. They found it “surprising” that most studies in the symposium concluded that long-distance dispersal was pervasive. However, it seems anything but surprising, given the methodology. Matthewian biogeography was based on a literal reading of the fossil record and concluded in favor of dispersal, and it is inevitable that a molecular biogeography which calibrates trees on a literal reading of the fossil record will reach the same conclusion.

Critics sometimes suggest that panbiogeography advocates ignoring the fossil record, but this is not true. Biogeographical analyses should incorporate all records, living and fossil, and aim at an integration of the two. Matthew’s (1915) maps provide a splendid example. Fossils provide a tremendous source of information on minimum ages and phylogeny of particular groups, and the broad change in form seen between fossils of, say, the Mesozoic and Cenozoic is, as the names suggest, dramatic evidence of evolution. However, a literal reading of the fossil record is unrealistic and reliance on it has led to the house of cards that is modern phylogeography. The consensus among many molecular biogeographers exists only because authors who use the same assumptions cite each other’s conclusions as supporting evidence.

Pennington et al. (2004b) discussed how to choose between using geological events or fossils in calibrating nodes on a tree. They concluded that the high frequency of long-distance dispersal “highlights the danger” of using geological events, especially “old” ones, because patterns will have been obscured. However, we only know that long-distance dispersal is frequent because the dates of many nodes in many papers (e.g., in Pennington et al., 2004a) are recent, and we only know they are recent because they were calibrated with fossils. This sort of reasoning is hardly convincing.

Critics have observed a “strong and persistent desire” (Near and Sanderson, 2004) and a “great thirst” (Graur and Martin, 2004) to know the divergence dates of clades and it seems a certain impatience has clouded judgment and led to rushed conclusions in many molecular studies. Good science requires a degree of caution and scepticism and systematists should constantly, critically examine the basic assumptions their methodology involves, rather than taking them for granted or sweeping them under the carpet.

With millions of species and now DNA sequencing, biology has an immense amount of information on differentiation in space; virtually infinitely more than geology, with its few hundred major minerals. But biology still lags a hundred years behind geology in all aspects of mapping (Heads, in press b) and the use of “chance dispersal” means biology has nothing to offer its sister science. Ideally, as in the work of Wegener (1924), biology should be able to make fundamental contributions to tectonics. Molecular phylogenetic work is invaluable, but the emphasis should be on intensive sampling phylogenetically and geographically. Detailed mapping of clades is essential to permit correlation of distributions with different events in earth history. This is not necessarily a straightforward process, as geological features such as major faults and fracture zones are reactivated repeatedly over long periods of time. However, in the long term this should be a more profitable line of inquiry than the current emphasis on calibrating molecular divergences with fossils.

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References

- Arbogast, B.S., Edwards, S.V., Wakeley, J., Beeli, P., Slowinski, J.B., 2002. Estimating divergence times from molecular data on phylogenetic and population genetic time scales. *Ann. Rev. Ecol. Syst.* 33, 707–740.
- Avice, J.C., 2000. *Phylogeography: the History and Formation of Species*. Harvard University Press, Cambridge, MA.
- Baldwin, B.G., Crawford, D.J., Francisco-Ortega, J., Kim, S.C., Sang, T., Stuessy, T.F., 1998. Molecular phylogenetic insights on the origin and evolution of oceanic island plants. In: Soltis, D.E., Soltis, P.S., Doyle, J.J. (Eds.), *Molecular Systematics of Plants II. DNA Sequencing*. Kluwer, Boston, pp. 410–441.
- Banford, H.M., Bermingham, E., Collette, B.B., McCafferty, S.S., 1999. Phylogenetic systematics of the *Scomberomorus regalis* (Teleostei: Scombridae) species group: molecules, morphology and biogeography of Spanish mackerels. *Copeia*, 1999, 596–613.
- Benton, M.J., Ayala, F.J., 2003. Dating the tree of life. *Science*, 300, 1698–1700.
- Bossuyt, F., Milinkovitch, M.C., 2001. Amphibians as indicators of early Tertiary ‘out-of-India’ dispersal of vertebrates. *Science*, 292, 93–95.
- Bremer, K., 2002. Gondwanan evolution of the grass alliance of families (Poales). *Evolution*, 56, 1374–1387.
- Briggs, J.C., 1974. Operation of marine zoogeographic barriers. *Syst. Zool.* 23, 248–256.

- Brochu, C.A., 2004. Calibration age and quartet divergence date estimation. *Evolution*, 58, 1375–1382.
- Buckley, T.R., Simon, C., Chambers, G.K., 2001. Phylogeography of the New Zealand cicada *Maoricicada campbelli* based on mitochondrial DNA sequences: ancient clades associated with Cenozoic environmental change. *Evolution*, 55, 1395–1407.
- Cifelli, R.L., Davis, B.M., 2003. Marsupial origins. *Science*, 302, 1899–1900.
- Conti, E., Rutschmann, F., Eriksson, T., Sytsma, K.J., Baum, D.A., 2004. Response to Robert G. Moyle 'Calibration of molecular clocks and the biogeographic history of Crypteroniaceae'. *Evolution*, 58, 1874–1876.
- Cowie, R.H., 1996. Pacific island land snails: relationships, origins and determinants of diversity. In: Keast, A., Miller, S.E. (Eds.), *The Origin and Evolution of Pacific Island Biotas, New Guinea to Eastern Polynesia: Patterns and Processes*. SPB Academic Publishing, Amsterdam, pp. 347–372.
- Craw, R.C., 1982. Phylogenetics, areas, geology and the biogeography of Croizat: a radical view. *Syst. Zool.* 31, 304–316.
- Craw, R.C., 1983. Panbiogeography and vicariance biogeography: are they truly different? *Syst. Zool.* 32, 431–438.
- Craw, R.C., 1985. Classic problems of southern hemisphere biogeography re-examined: panbiogeographic analysis of the New Zealand frog *Leiopelma*, the ratite birds and *Nothofagus*. *Zeit. Zool. Syst. Evol.* 23, 1–10.
- Craw, R.C., Grehan, J.R., Heads, M.J., 1999. *Panbiogeography: Tracking the History of Life*. Oxford University Press, New York.
- Croizat, L., 1952. *Manual of Phytogeography*. Junk, The Hague.
- Croizat, L., 1958. *Panbiogeography*. Published by the Author, Caracas.
- Croizat, L., 1964. *Space, Time, Form: the Biological Synthesis*. Published by the Author, Caracas.
- Croizat, L., 1975. Biogeografía analítica y sintética ('Panbiogeografía') de las Américas. *Bol. Acad. Cienc. Fis., Mat. Nat. [Caracas]* 35, 103–106. [Also published as *Bibl. Acad. Cienc. Fis., Mat. Nat. [Caracas]*, 15 and 16, 1976].
- Croizat, L., 1977. Carlos Darwin y sus teorías. *Bol. Acad. Cienc. Fis., Mat. Nat. [Caracas]* 37 (113), 15–90.
- Croizat, L., 1981. Vicariance/vicariism, panbiogeography, 'vicariance biogeography', etc. a clarification. *Syst. Zool.* 31, 291–304.
- Cunningham, C.W., Collins, T.M., 1994. Developing model systems for molecular biogeography: vicariance and interchange in marine invertebrates. In: Schierwater, B.S., Streit, B.B., Wagner, G.P., DeSalle, R. (Eds.), *Molecular Ecology and Evolution: Approaches and Applications*. Birkhäuser, Basel, pp. 405–433.
- Darlington, P.D., 1957. (1966 Reprint). *Zoogeography: the Geographical Distribution of Animals*. Wiley, New York.
- Darwin, C., 1971 [1882]. *On the Origin of Species*, 6th edn. Dent, London.
- Donovan, S.K., Paul, C.R.C. (Eds.), 1998a. *The Adequacy of the Fossil Record*. Wiley, Chichester, UK.
- Donovan, S.K., Paul, C.R.C., 1998b. Introduction: adequacy vs. incompleteness. In: Donovan, S.K., Paul, C.R.C. (Eds.), *The Adequacy of the Fossil Record*. Wiley, Chichester, UK, p. ix–x.
- Douady, C.J., Catzeflis, F., Kao, D.J., Springer, M.S., Stanhope, M.J., 2002. Molecular evidence for the monophyly of Tenrecidae (Mammalia) and the timing of the colonization of Madagascar by Malagasy tenrecs. *Mol. Phylog. Evol.* 22, 357–363.
- Dugdale, J.S., Fleming, C.A., 1978. New Zealand cicadas of the genus *Maoricicada* (Homoptera: Tibicinidae). *NZ. J. Zool.* 5, 295–340.
- Ebach, M., Humphries, C.J., 2002. Cladistic biogeography and the art of discovery. *J. Biogeog.* 29, 427–444.
- Ehrendorfer, F., Samuel, R., 2000. Comments on S. B. Hoot's interpretation of Southern Hemisphere relationships in *Anemone* (Ranunculaceae) based on molecular data [Am. J. Bot. 2000; 87(6, Suppl.), 154–155]. *Taxon*, 49, 781–784.
- Engel, M.S., 2000. A new interpretation of the oldest fossil bee (Hymenoptera: Apidae). *Am. Mus. Nov.* 3296, 1–11.
- Ernst, C.H., Barbour, R.W., 1989. *Turtles of the World*. Smithsonian Institution Press, Washington, DC.
- Fishbein, M., Hibsich-Jetter, C., Soltis, D.E., Hufford, L., 2001. Phylogeny of Saxifragales (Angiosperms, Eudicots): analysis of a rapid, ancient radiation. *Syst. Biol.* 50, 817–847.
- Galen, 1916. *On the Natural Faculties*. Transl. Brock, A.J. Heinemann, London.
- Gao, K., Shubin, N.H., 2003. Earliest known crown group salamanders. *Nature*, 422, 424–426.
- Gee, H., 1999. *In Search of Deep Time: Beyond the Fossil Record to a New History of Life*. Free Press, New York.
- Givnish, T.J., Millam, K.C., Evans, T.M., Hall, J.C., 2004. Ancient vicariance or recent long-distance dispersal? Inferences about phylogeny and South American-African disjunctions in Rapateaceae and Bromeliaceae based on *ndhF* sequence data. *Int. J. Plant Sci.* 165(Suppl. 4), S35–S54.
- Givnish, T.J., Renner, S.S. (Eds.), 2004a. Tropical intercontinental disjunctions. *Int. J. Plant Sci.* 165(4 Suppl.), 1–6.
- Givnish, T.J., Renner, S.S., 2004b. Tropical intercontinental disjunctions: Gondwana breakup, immigration from the boreal tropics, and transoceanic dispersal. *Int. J. Plant Sci.* 165 (4 Suppl.), S1–S6.
- Glynn, P.W., 1972. Observations on the ecology of the Caribbean and Pacific coasts of Panama. *Bull. Biol. Soc. Washington*, 2, 13–30.
- Glynn, P.W., 1982. Coral communities and their modifications relative to past and prospective Central American seaways. *Adv. Mar. Biol.* 19, 91–132.
- Gould, S., 1989. *The Burgess Shale and the Nature of History*. Penguin, London.
- Graur, D., Martin, W., 2004. Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends Genet.* 20, 80–86.
- Hall, R., 2001. Cenozoic reconstructions of SE Asia and the SW Pacific: changing patterns of land and sea. In: Metcalfe, I., Smith, J.M.B., Morwood, M., Davidson, I.D. (Eds.), *Faunal and Floral Migrations and Evolution of SE Asia and Australia*. Balkema, Rotterdam, pp. 35–56.
- Heads, M., 1985. Biogeographic analysis of *Nothofagus* (Fagaceae). *Taxon*, 34, 474–492.
- Heads, M., 1990. Integrating earth and life sciences in New Zealand natural history: the parallel arcs model. *NZ. J. Zool.* 16, 549–586.
- Heads, M., 1999. Vicariance biogeography and terrane tectonics in the South Pacific: an analysis of the genus *Abrotanella* (Compositae), with a new species from New Zealand. *Biol. J. Linn. Soc.* 67, 391–432.
- Heads, M., 2001. Birds of paradise, biogeography and ecology in New Guinea: a review. *J. Biogeog.* 28, 893–927.
- Heads, M., 2002. Birds of paradise, vicariance biogeography and terrane tectonics in New Guinea. *J. Biogeog.* 29, 261–284.
- Heads, M., 2003. Ericaceae in Malesia: vicariance biogeography, terrane tectonics and ecology. *Telopea*, 10, 311–449.
- Heads, M., 2004. What is a node? *J. Biogeog.* 31, 1883–1891.
- Heads, M., in press, a. Towards a panbiogeography of the seas. *Biol. J. Linn. Soc.* in press.
- Heads, M., in press, b. History and philosophy of panbiogeography. In: Morrone, J. (Ed.), *Biogeography in Latin America*. Universidad Nacional Autónoma de México, Mexico City.
- Heenan, P.B., Mitchell, A.D., Koch, M., 2002. Molecular systematics of the New Zealand *Pachycladon* (Brassicaceae) complex: generic circumscription and relationships to *Arabidopsis* sens. lat. and *Arabis* sens. lat. *NZ. J. Bot.* 40, 543–562.
- Hennig, W., 1966. *Phylogenetic Systematics*. University of Illinois Press, Urbana, IL.
- Hoffmann, H., 2000. The rise of life on earth: Messel – window on an ancient world. *Nat. Geog. Mag.* 2000(2), 34–51.
- Hofman, A., Maxson, L.R., Arntzen, J.W., 1991. Biochemical evidence pertaining to the taxonomic relationships within the family Chameleoniidae. *Amphibia-Reptilia*, 12, 245–265.

- Humphries, C.J., 1981a. Biogeographical methods and the southern beeches. In: Forey, P. (Ed.), *The Evolving Biosphere*. Cambridge University Press, Cambridge, UK, pp. 283–297.
- Humphries, C.J., 1981b. Biogeographical methods and the southern beeches (Fagaceae: *Nothofagus*). In: Funk, V., Brooks, D. (Eds.), *Advances in Cladistics*. New York Botanical Garden, New York, pp. 177–207.
- Humphries, C.J., 2000. Form, space, time: which comes first? *J. Biogeog.* 27, 11–15.
- Hurr, K.A., Lockhart, P., Heenan, P.B., Penny, D., 1999. Evidence for the recent dispersal of *Sophora* (Leguminosae) around the Southern Oceans: molecular data. *J. Biogeog.* 26, 565–577.
- Hutton, F.J., 1872. On the geographic relations of the New Zealand fauna. *Trans. NZ. Inst.* 5, 227–256.
- Jackson, J.B.C., Jung, P., Coates, A.G., Collins, L.S., 1993. Diversity and extinction of tropical American mollusks and closure of the Isthmus of Panama. *Science*, 260, 1624–1626.
- de Jong, R., Congdon, T.C.E., 1993. The montane butterflies of the eastern Afrotropics. In: Lovett, J.C., Wasser, S.K. (Eds.), *Biogeography and Ecology of the Rain Forests of Eastern Africa*. Cambridge University Press, Cambridge, UK, pp. 133–172.
- Kearey, P., Vine, F.J., 1996. *Global Tectonics*, 2nd edn. Blackwell, Oxford.
- Kimball, R.T., Crawford, D.J., Smith, E.B., 2003. Evolutionary processes in the genus *Coreocarpus*: insights from molecular phylogenetics. *Evolution*, 57, 52–61.
- Klak, C., Reeves, G., Hedderson, T., 2004. Unmatched tempo of evolution in Southern African semi-desert ice plants. *Nature*, 427, 63–65.
- Klaver, C.J.J., Böhme, W., 1986. Phylogeny and classification of the Chamaeleonidae (Sauria) with special reference to hemipenis morphology. *Bonn Zool. Monogr.* 22, 1–64.
- Klaver, C.J.J., Böhme, W., 1997. *Chamaeleonidae* (Animal Kingdom, 112). Walter de Gruyter, Berlin.
- Knowlton, N., 1993. Sibling species in the sea. *Ann. Rev. Ecol. Syst.* 24, 189–216.
- Knowlton, N., Weigt, L.A., 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proc. Roy. Soc. London B*, 265, 2257–2263.
- Koch, M.A., Haubold, B., Mitchell-Olds, T., 2000. Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in *Arabidopsis*, *Arabis*, and related genera (Brassicaceae). *Mol. Biol. Evol.* 17, 1483–1498.
- Lockhart, P.J., Mclenachan, P.A., Havell, D., Glenney, D., Huson, D., Jensen, U., 2001. Phylogeny, radiation, and transoceanic dispersal of New Zealand alpine buttercups: molecular evidence under split decomposition. *Ann. Miss. Bot. Gard.* 88, 462–477.
- Luo, Z.-X., Ji, Q., Wible, J.R., Yuan, C.-X., 2003. An early Cretaceous tribosphenic mammal and metatherian evolution. *Science*, 302, 1934–1940.
- Magallón, S.A., 2004. Dating lineages: molecular and paleontological approaches to the temporal framework of clades. *Int. J. Plant Sci.* 165(4, Suppl.), S7–S21.
- Matthew, W.D., 1915. Climate and evolution. *Ann. NY Acad. Sci.* 24, 171–318.
- Mayr, E., 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.
- Mayr, G., 2004. Old World fossil record of modern-type hummingbirds. *Science*, 304, 861–864.
- McCall, R.A., 1997. Implications of recent geological investigations of the Mozambique Channel for the mammalian colonization of Madagascar. *Proc. Bio. Sci.* 264, 663–665.
- McDaniel, S.F., Shaw, A.J., 2003. Phylogeographic structure and cryptic speciation in the trans-Antarctic moss *Pterobryum mnioides*. *Evolution*, 57, 205–215.
- Muss, A., Robertson, D.R., Stepien, C.A., Wirtz, P., Bowen, B.W., 2001. Phylogeography of *Ophioblennius*: the role of ocean currents and geography in reef fish evolution. *Evolution*, 55, 561–572.
- Near, T.J., Sanderson, M.J., 2004. Assessing the quality of molecular divergence time estimates by fossil calibrations and fossil-based model selection. *Phil. Trans. R. Soc. Lond. B*, 359, 1477–1483.
- Nelson, G., 2004. Cladistics: its arrested development. In: Williams, D.M., Forey, P.L. (Eds.), *Milestones in Systematics: the Development of Comparative Biology*. Taylor & Francis, London, pp. 127–147.
- Nelson, G., Ladiges, P.Y., 2001. Gondwana, vicariance biogeography, and the New York school revisited. *Aust. J. Bot.* 49, 389–409.
- Norman, J.E., Ashley, M.F., 2000. Phylogenetics of Perissodactyla and tests of the molecular clock. *J. Mol. Evol.* 50, 11–21.
- Pennington, R.T., Cronk, Q.C.B., Richardson, J.A. (Eds.), 2004a. Plant phylogeny and the origin of major biomes. *Phil. Trans. R. Soc. Lond. B* 359 (1450).
- Pennington, R.T., Cronk, Q.C.B., Richardson, J.A., 2004b. Introduction and synthesis: plant phylogeny and the origin of major biomes. *Phil. Trans. R. Soc. Lond. B*, 359, 1455–1464.
- Perrie, L.R., Brownsey, P.J., Lockhart, P.J., Brown, E.A., Large, M.F., 2003. Biogeography of temperate Australasian *Polystichum* ferns as inferred from chloroplast sequence and AFLP. *J. Biogeog.* 30, 1729–1736.
- Philpson, W.R., 1970. Floristics of Rarotonga. In: Fraser, R. (Ed.), *The Cook Bicentenary Expedition in the Southwestern Pacific*. Royal Society of New Zealand (Bulletin 8). Wellington, pp. 49–54.
- Raxworthy, C.J., Forstner, M.R.J., Nussbaum, R.A., 2002. Chameleon radiation by oceanic dispersal. *Nature*, 415, 784–787.
- Renner, S.S., Clausen, G., Meyer, K., 2001. Historical biogeography of Melastomataceae: the roles of Tertiary migration and long-distance dispersal. *Am. J. Bot.* 88, 1290–1300.
- Renner, S.S., Foreman, D.B., Murray, D., 2000. Timing transantarctic disjunctions in the Atherospermataceae (Laurales): evidence from coding and noncoding chloroplast sequences. *Syst. Bot.* 49, 579–591.
- Renner, S.S., Meyer, K., 2001. Melastomeae come full circle: biogeographic reconstruction and molecular clock dating. *Evolution*, 55, 1315–1324.
- Richards, C.M., Moore, W.S., 1996. A phylogeny for the African tree frog family Hyperoliidae based on mitochondrial rDNA. *Mol. Phylogen. Evol.* 5, 522–532.
- Richardson, J.E., Pennington, R.T., Pennington, T.D., Hollingsworth, P.M., 2001a. Rapid diversification of a species-rich genus of Neotropical rain forest trees. *Science*, 293, 2242–2245.
- Richardson, J.E., Weitz, F.M., Fay, M.F., Cronk, Q.C.B., Linder, H.P., Reeves, G., Chase, M.W., 2001b. Rapid and recent origin of species richness in the Cape flora of South Africa. *Nature*, 412, 181–183.
- Rieppel, O., 2002. A case of dispersing chameleons. *Nature*, 415, 744–745.
- Rodriguez-Trelles, F., Tarrío, R., Ayala, F.J., 2004. Molecular clocks: whence and whither? In: Donoghue, P.C.J., Smith, M.P. (Eds.), *Telling the Evolutionary Time: Molecular Clocks and the Fossil Record*. CRC Press, Boca Raton, FL, pp. 5–26.
- Rosen, B., 1988. Progress, problems and patterns in the biogeography of reef corals and other tropical marine organisms. *Helgoländ. Meeres.* 40, 269–301.
- Sanmartín, I., Ronquist, F., 2004. Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. *Syst. Biol.* 53, 216–243.
- Savin, S.M., Douglas, R.G., 1985. Sea level, climate, and the central American land bridge. In: Stehli, F.G., Webb, S.D. (Eds.), *The Great American Biotic Interchange*. Plenum, New York, pp. 303–324.
- Seiffert, E.R., Simons, E.L., Attia, Y., 2003. Fossil evidence for an ancient divergence of lorises and galagos. *Nature*, 422, 421–423.
- Shaul, S., Graur, D., 2002. Playing chicken (*Gallus gallus*): Methodological inconsistencies of molecular divergence date estimates due to secondary calibration points. *Gene*, 300, 59–61.

- Smitsen, R.D., Garnock-Jones, P.J., Chambers, G.K., 2003. Phylogenetic analysis of ITS sequences suggests a Pliocene origin for the bipolar distribution of *Scleranthus* (Caryophyllaceae). *Aust. Syst. Bot.* 16, 301–313.
- Smith, A.B., Peterson, K.J., 2002. Dating the time of origin of major clades: molecular clocks and the fossil record. *Ann. Rev. Earth. Planet. Sci.* 30, 65–88.
- Sparks, J.S., 2004. Molecular phylogeny and biogeography of the Malagasy and South Asian cichlids (Teleostei: Perciformes: Cichlidae). *Mol. Phylog. Evol.* 30, 599–614.
- Sparks, J.S., Smith, W.L., in press. Phylogeny and biogeography of cichlid fishes (Teleostei: Perciformes: Cichlidae): a multilocus approach to recovering deep intrafamilial divergences and the cichlid sister group. *Cladistics*, 20, 1–17.
- Stehli, F.G., Webb, S.D., 1985. *The Great American Biotic Interchange*. Plenum, New York.
- Swenson, U., 1995. Systematics of *Abrotanella*, an amphipacific genus of Asteraceae (Senecioneae). *Plant Syst. Evol.* 197, 149–193.
- Swenson, U., Bremer, K., 1997. Pacific biogeography of the Asteraceae genus *Abrotanella* (Senecioneae, Blennospermatineae). *Syst. Bot.* 22, 493–508.
- Townsend, T., Larson, A., 2002. Molecular phylogenetics and mitochondrial genomic evolution in the Chameleonidae (Reptilia, Squamata). *Mol. Phylog. Evol.* 23, 22–36.
- Turk, F.A., 1964. Form, size, macromutation and orthogenesis in the Arachnida: an essay. *Ann. Natal Mus.* 16, 236–255.
- Van Tuinen, M., Hadly, E.A., 2004. Error in estimation of rate and time inferred from the early amniote fossil record and avian molecular clocks. *J. Mol. Evol.* 59, 267–276.
- Vences, M., Kosuch, J., Glaw, F., Veith, M., 2003. Molecular phylogeny of hyperoliid tree frogs: biogeographic origin of Malagasy and Seychellean taxa and reanalysis of familial paraphyly. *J. Zool. Syst. Evol. Res.* 41, 205–215.
- Verboom, G.A., Linder, H.P., Stock, W.D., 2003. Phylogenetics of the grass genus *Ehrharta*: evidence for radiation in the summer-arid zone of the South African Cape. *Evolution*, 57, 1008–1021.
- Voelker, G., 1999. Dispersal, vicariance, and clocks: historical biogeography and speciation in a cosmopolitan passerine genus (*Anthus*, Motacillidae). *Evolution*, 53, 1536–1552.
- Wagstaff, S.J., Bayly, M.J., Garnock-Jones, P.J., Albach, D.C., 2002. Classification, origin, and diversification of the New Zealand hebes (Scrophulariaceae). *Ann. Missouri Bot. Gard.* 89, 38–63.
- Wallace, A.R., 1876. *The Geographical Distribution of Animals*. Macmillan, London.
- Wallace, A.R., 1998 [1881]. *Island Life*. Prometheus, New York.
- Wallis, G.P., Trewick, S.A., 2001. Finding fault with vicariance: a critique of Heads (1998). *Syst. Biol.* 50, 602–609.
- Waters, J.M., Roy, M.S., 2003. Global phylogeography of the fissiparous sea-star genus *Coscinasterias*. *Mar. Biol.* 142, 185–191.
- Waters, J.M., Roy, M.S., 2004. Out of Africa: the slow train to Australasia. *Syst. Biol.* 53, 18–24.
- de Weerd, W.H., 1990. Discontinuous distribution of the tropical West Atlantic hydrocoral *Millepora squarrosa*. *Beaufortia*, 41, 195–203.
- Wegener, A., 1924. *The Origin of Continents and Oceans*. Methuen, London.
- White, B.N., 1986. The isthmian link, antitropicality and American biogeography: distributional history of the Atherinopsinae (Pisces: Atherinidae). *Syst. Zool.* 35, 176–194.
- Wikström, N., Savolainen, V., Chase, M.W., 2001. Evolution of the angiosperms: calibrating the family tree. *Proc. Roy. Soc. London B*, 268, 2211–2220.
- Williams, D.M., Ebach, M.C., 2004. The reform of palaeontology and the rise of biogeography – 25 years after ‘ontogeny, phylogeny, paleontology and the biogenetic law’ (Nelson, 1978). *J. Biogeog.* 31, 685–712.
- Winkworth, R.C., Grau, J., Robertson, A.W., Lockhart, P.J., 2002. The origins and evolution of the genus *Myosotis* L. (Boraginaceae). *Mol. Phylog. Evol.* 24, 180–193.