

New dates and new rates for divergence across the Isthmus of Panama

Nancy Knowlton^{1*} and Lee A. Weigt^{2†}

¹Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama

²Field Museum of Natural History, Roosevelt Road at Lake Shore Drive, Chicago, IL 60605, USA

Sister species separated by the Isthmus of Panama have been widely used to estimate rates of molecular evolution. These estimates are based on the assumption that geographic isolation occurred nearly simultaneously for most taxa, when connections between the Caribbean and eastern Pacific closed approximately three million years ago. Here we show that this assumption is invalid for the only genus for which many taxa and multiple genetic markers have been analysed. Patterns of divergence exhibited by allozymes and the mitochondrial COI gene are highly concordant for 15 pairs of snapping shrimp in the genus *Alpheus*, indicating that they provide a reasonable basis for estimating time since cessation of gene flow. The extent of genetic divergence between pairs of sister species varied over fourfold. Sister species from mangrove environments showed the least divergence, as would be expected if these were among the last habitats to be divided. Using this pair yields a rate of sequence divergence of 1.4% per one million years, with implied times of separation for the 15 pairs of 3–18 million years ago. Many past studies may have overestimated rates of molecular evolution because they sampled pairs that were separated well before final closure of the Isthmus.

Keywords: allozymes; *Alpheus*; COI; molecular clock; mtDNA; Panama

1. INTRODUCTION

The Isthmus of Panama represents a complete, relatively recent, and well-dated barrier across what was once a large neotropical marine environment (Farrell *et al.* 1995; Coates & Obando 1996). Sister species separated by the Isthmus have thus provided an important tool for estimating rates and patterns of molecular evolution for many marine groups (Lessios 1979, 1998; Vawter *et al.* 1980; Martin *et al.* 1992; Knowlton *et al.* 1993; Bermingham & Lessios 1993; Collins 1996; Bermingham *et al.* 1997; Sturmbauer *et al.* 1996; Hart *et al.* 1997; Schubart *et al.* 1998).

Studies to test the constancy and estimate the rate of molecular evolution are based on the assumption that most transisthmian sister-species pairs were separated at roughly the same time by final closure of the connection between the Caribbean and the eastern Pacific (Collins 1996), approximately three million years ago (Ma) (Coates & Obando 1996). Recent molecular and fossil studies suggest that this assumption may be invalid (Knowlton *et al.* 1993; Jackson *et al.* 1993), but the significance of these concerns remains unclear (Cunningham & Collins 1994). The continuing use of the Isthmus as a basis for estimating rates of molecular evolution (see, for example, Burton & Lee 1994; Sturmbauer *et al.* 1996; Hart *et al.* 1997; Chenoweth *et al.* 1998; Metz *et al.* 1998;

Schubart *et al.* 1998) makes resolution of the timing of transisthmian divergences an important task.

Here we report allozyme and mtDNA divergences for 15 transisthmian sister-species pairs in the snapping shrimp genus *Alpheus*. All are shallow-water forms, but two pairs are restricted to mangroves, which were likely to be the last marine habitats separated by the rising Isthmus. This transisthmian molecular data set is unique, in that no other genus analysed to date contains more than two sister-species pairs (Bermingham *et al.* 1997; Schubart *et al.* 1998), and with few exceptions (Knowlton *et al.* 1993; Bermingham & Lessios 1993; Metz *et al.* 1998), independent measures of genetic divergence (such as those based on mitochondrial and nuclear genes or gene products) are not available. Multiple taxa and independent measures of genetic divergence are essential, because a single gene might misrepresent the species tree, and a single species might misrepresent the general biogeographic history of a region.

2. MATERIALS AND METHODS

(a) Taxa

The taxonomic literature suggests approximately 20 possible transisthmian sister-species pairs in the genus *Alpheus* (Kim & Abele 1988). In some cases these are currently recognized as distinct at the species level, and in other cases not. This inconsistency is a taxonomic artefact; all should be recognized as distinct species because of fixed genetic differences and reproductive incompatibilities (Knowlton *et al.* 1993; this paper).

Shrimp were initially identified by using the keys published by Chace (1972) and Kim & Abele (1988). In the course of our

* Author for correspondence (knowlton@naos.si.edu).

† Present address: CMMID, Virginia Tech, 1410 Prices Fork Road, Blacksburg, VA 24061-0342, USA.

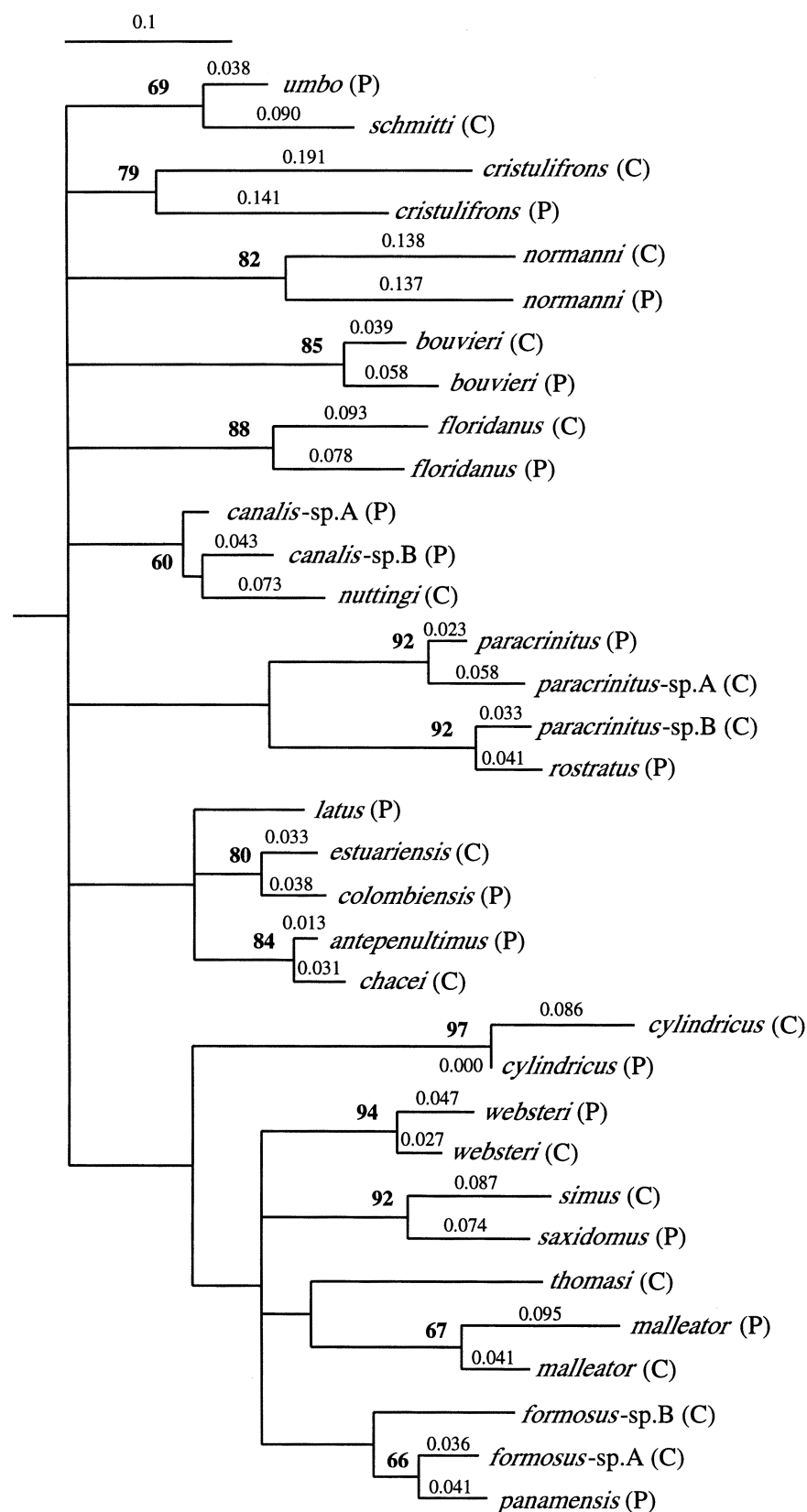


Figure 1. COI-based maximum likelihood approximation (Puzzle 4.0) (Strimmer *et al.* 1997) phylogeny of *Alpheus* taxa with possible transisthmian sister species. Morphological (see Knowlton *et al.* 1993) and molecular assignments of sister species were entirely consistent. Likelihood approximation reliability estimates are shown in bold to the left of the 15 transisthmian pairs. Branch lengths are shown above each Caribbean (C) and Pacific (P) pair member.

collections we discovered several additional, undescribed species that resembled taxa with close transisthmian relatives; these sibling species were also included (see Knowlton & Mills 1992; Knowlton *et al.* 1993). All shrimp were collected from the Caribbean and Pacific coasts of Panama, typically from the intertidal zone and in no case from depths of more than 20 m. In total we collected over 2000 individuals from a variety of habitats.

Correct identification of transisthmian sister species is essential for estimating rates of molecular evolution, and can be a problem in highly diverse groups like *Alpheus* (Lessios 1998). Many transisthmian sister-species pairs closely resemble each other in morphology and colour pattern, and do not resemble other *Alpheus* from the eastern Pacific or western Atlantic. This allows one to assign sister-species pairs reliably without resorting to molecular data. In several cases two sibling species occur in

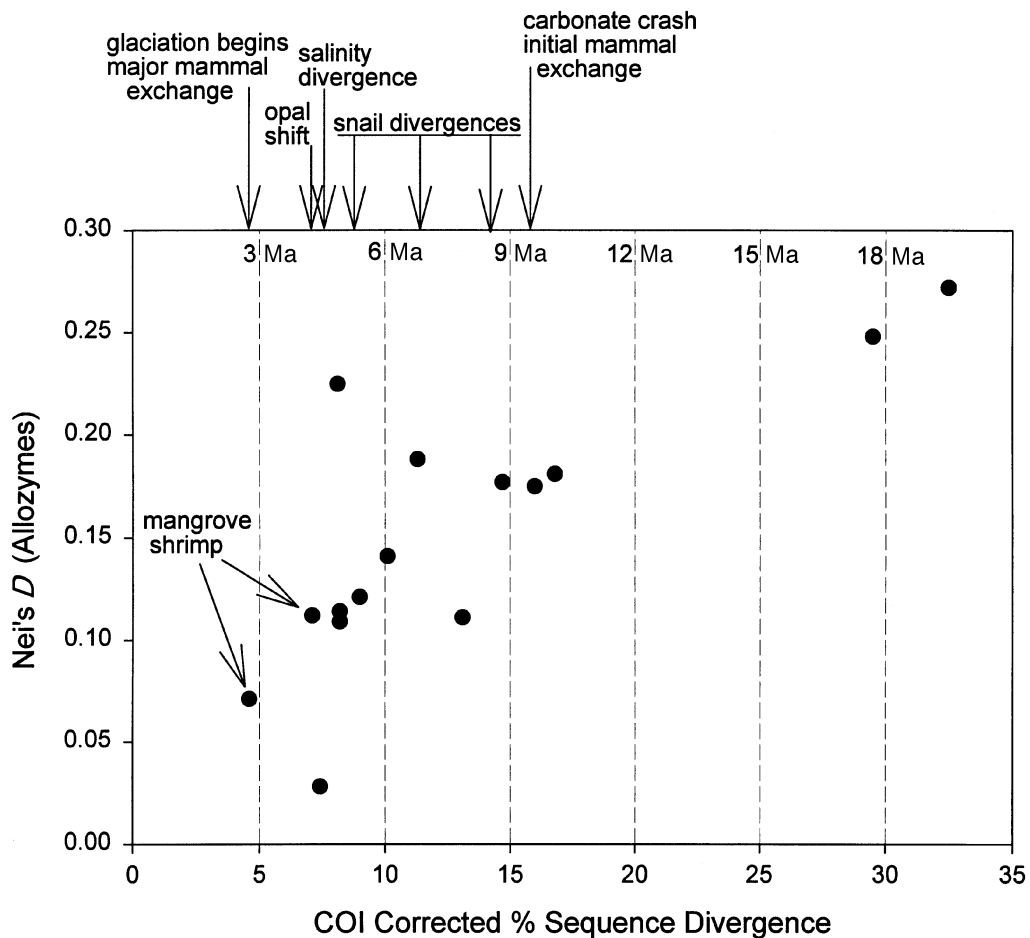


Figure 2. Relation between corrected per cent sequence divergence and allozyme differentiation (Nei's D) for 15 independent pairs of transisthmian sister species (figure 1). Maximum likelihood divergence estimates were corrected for among-site rate variation by means of a four-category gamma distribution. Just below the top axis are approximate dates of divergence based on COI data, with the assumption that the most similar pair diverged at approximately 3 Ma. Various biological (Collins 1989; Cunningham & Collins 1994) and geological (table 5 of Farrell *et al.* (1995)) events related to the rise of the Isthmus, whose timing has been independently estimated based on the geological record, are positioned above the top axis to show their relationship to the timing of shrimp divergences based on molecular rate calibrations. The two most distant pairs appear to be the survivors of lineages that diverged about the time the Tethyan connection between Atlantic and Pacific was severed.

one or both oceans, but in these cases sister species are also readily identified by means of behavioural tests or subtle differences in morphology and colour (Knowlton *et al.* 1993). However, we excluded potential transisthmian pairs involving the Caribbean *A. armillatus* and *A. heterochaelis* complexes because we were not confident that sister species could be assigned or collected owing to the large number of sibling species in these groups.

(b) Analyses

After identification, living shrimp were frozen in liquid N_2 , or placed directly in a $-80^\circ C$ ultracold freezer for storage before processing. For all pairs of taxa, we characterized at least 11 and typically 15–16 allozyme loci by using conventional starch gel electrophoresis, and sequenced 564 base pairs (bp) of the mtDNA cytochrome oxidase I (COI) gene (GenBank accession numbers U02002–U02018, AF097858–AF097873) (see Knowlton *et al.* (1993) for details). We used the same methods as in our earlier analyses, except that cycle-sequencing reactions were performed with a dRhodamine kit (PE-ABI) or

a Thermosequenase kit (Amersham) at 10 μl volume (modified to include halfTERM (Genpak)), following manufacturers' instructions, and run on an ABI377 automated DNA sequencer. Sequences were trimmed and aligned by means of Sequencher (v3.0, Genecodes), and analysed with the Puzzle 4.0 (Strimmer *et al.* 1997) maximum likelihood approximation.

3. RESULTS

As before (Knowlton *et al.* 1993), transisthmian sister species identified by traditional morphological criteria (Chace 1972; Kim & Abele 1988; Knowlton & Mills 1992) were confirmed by phylogenies based on the mitochondrial COI gene (figure 1). Doubling the sample size from seven to 15 pairs strengthened our earlier conclusion that both measures of genetic divergence (allozymes and mtDNA) varied widely across the pairs of taxa in a concordant fashion (figure 2).

The most parsimonious interpretation of these results is that time of isolation varied widely among these pairs of

taxa. The magnitude of variability in mtDNA divergence between pairs is inconsistent with simultaneous isolation and clock-like divergence based on an analysis of the nucleotide changes shown in figure 1: the statistic $R(n-1)$ (where R is the mean:variance of number of nucleotide changes, number of changes equals branch lengths multiplied by number of base pairs, and n is the number of lineages) (Goldman 1994) is significantly non-random for all sites and third positions only, both for all taxa and when the two most dissimilar pairs (figure 2) are excluded ($p < 0.001$). Concordance between the two independent measures of divergence (figure 2) also points strongly to non-simultaneous isolation as the most parsimonious explanation for this pattern (Knowlton *et al.* 1993; Bermingham & Lessios 1993; Collins 1996).

Non-simultaneous divergence is also supported by the fact that species pairs restricted to offshore islands or deeper habitats are typically more divergent than those found along the mainland (Knowlton *et al.* 1993; this paper). Moreover, the smallest divergence values were seen for shrimp pairs from mangroves (figure 2), as expected if these were the last habitats separated by the rising Isthmus.

If the 15 transisthmian sister species did not diverge simultaneously, then the lowest mtDNA divergence value, rather than the average divergence of all shrimp pairs, provides a better estimate of the rate of molecular evolution. This rate can then be used to reconstruct the timing of isolation of more divergent pairs (figure 2). The use of a geological estimate of final closure of the connection between the Caribbean and eastern Pacific approximately 3 Ma (Coates & Obando 1996), together with a gamma correction for COI divergences, yielded estimated dates of divergence ranging from 3 Ma (most similar mangrove pair) to ca. 18 Ma (two most divergent pairs) (figure 2).

Divergences between most transisthmian pairs fell within a 3–9-million-year range. This interval is bracketed at one end by the first crossing of mammals between North and South America, and at the other by the onset of glaciation and massive mammal interchange (figure 2), lending credence to the reliability of the rate calibrations. Similarly, a pulse of divergences about 4.5 Ma coincides with several oceanographic measures of basin isolation (events summarized by Farrell *et al.* (1995)). The two most highly divergent pairs fall outside this interval, however, and presumably represent a pre-Isthmian event (see below).

4. DISCUSSION

(a) *Caveats*

Comparison of true sister species is fundamental to our conclusion that most transisthmian sister taxa are considerably older than three million years, because aberrantly high divergence values could be caused by misassigning sister-species pairs or failing to collect them. The concordance of morphological, behavioural and molecular data sets (Knowlton *et al.* 1993; this paper) suggests that closest relatives among the taxa we collected have been identified correctly. Kim & Abele's (1988) compilation indicates no potential transisthmian sister species involving eastern Pacific shrimp with ranges that do not include Panama, and based on our collections their monograph is very

complete. Although monographs for the Caribbean are less comprehensive for *Alpheus*, our own Caribbean collections outside Panama (albeit limited) revealed divergences between presumed conspecifics of less than 1.5% (Cunningham & Collins 1994; Knowlton & Weigt 1997; N. Knowlton and L. A. Weigt, unpublished data). Thus failure to compare closest transisthmian relatives does not seem a likely explanation for the overall pattern we observed.

A second potential source of error is our assumption that connections between the Caribbean and eastern Pacific ended about 3 Ma. A few authors have suggested leakage or a final breach of the Isthmus as recently as 2 Ma (Cronin & Dowsett 1996). If we assign a date of 2 Ma to the divergence of the most similar mangrove species, then divergence times for the remaining pairs by extrapolation would still range from 2–14 Ma, with over half of all pairs having divergences of more than four million years. However, empirical support for a biologically important breach of the Isthmus this recently is thin. Indeed, Cronin & Dowsett (1996) suggest that the barrier began to have substantial effects on surface water flow by 3.5 Ma, but with a reopening of previously closed connections between 3.1 and 2.8 Ma due to especially high global sea level. This scenario would also match the temporal pattern of divergences suggested by the molecular data without having to invoke a 2 Ma breach of the Isthmus.

Finally, our analyses could be confounded by rate inconstancy, particularly if all genetically similar shrimp pairs belonged to clades that were on average slowly evolving, and all genetically divergent shrimp pairs belonged to rapidly evolving clades. This does not seem to be true, however. We could detect no significant deviations in rates based on pairwise comparisons (Wu & Li 1985) once Bonferroni corrections for the number of simultaneous comparisons were applied (smallest $p < 0.001$; that required for significance with multiple tests is $p < 0.0002$). Morrison (1997) also found that *Alpheus* (but not *Synalpheus*) exhibited rate constancy in a study of transisthmian taxa. Thus, although we cannot rule out minor differences in rates of molecular evolution within the genus, all available data suggest that rate variation is not a major contributor to the general pattern observed in *Alpheus*.

(b) *Implications and generality*

There are several processes that could result in non-simultaneous divergence times between transisthmian sister taxa (Lessios 1998). The most obvious of these is variation in the timing of severance of gene flow across the Isthmian region. Although such variation could be entirely stochastic, ecological differences between the least and most divergent pairs suggest that at least some of the variation is ecologically based. Various aspects of larval behaviour and physiology might contribute to such a pattern. Grosberg (1982), for example, documented that larval depth stratification mirrored that of adult populations on a very fine scale in barnacles. A comparable situation in snapping shrimp could lead to differences in the timing of the cessation of gene flow across the Isthmus if shallow-water larvae were less likely to be blocked by reduced water flow than deeper-dwelling larvae. Larvae from adults restricted to offshore reef environments might

also be more likely to avoid bodies of turbid, lower-salinity water over the rising Isthmus. Future experiments with larvae of extant transisthmian species having different divergence values could be informative in this regard.

Extinction can also have major effects on biogeographic patterns (Vermeij 1991; Cunningham & Collins 1998), including those associated with the Isthmus of Panama (Cunningham & Collins 1994; Lessios 1998). Consider, for example, the extant quartet of sibling species belonging to the *A. paracrinitus*–*A. rostratus* complex (figure 1). This quartet consists of two clades, each with a transisthmian pair, but they are so similar morphologically that they were only recently distinguished (Kim & Abele 1988). If the Caribbean member of one clade and the Pacific member of the other clade had gone extinct, it would lead to an apparent pair of much more divergent transisthmian sister species. Such differential extinction patterns are not unexpected, considering the oceanographic differences between the Caribbean and the eastern Pacific. Interestingly, the amount of divergence between these two clades (data in Knowlton *et al.* (1993)) is very similar to that observed for the two most divergent extant pairs in this study. The cause of three independent divergences at about 18 Ma (figure 2) is unclear, but may be related to oceanographic changes associated with the closure of the Tethyan seaway at about that time.

No evidence for very recent speciation (less than 2 Ma) within either ocean is provided by these data; there are no sympatric sibling-species pairs with divergences less than those exhibited by the mangrove transisthmian taxa. This is similar to the pattern observed for Caribbean benthic foraminifera, which show comparatively few originations after 3.5 Ma (L. S. Collins *et al.* 1996). However, radiations in the past two million years have been suggested for several other groups, especially in the Pacific, based on molecular (see, for example, McMillan & Palumbi 1995; Palumbi 1996) and fossil (Jackson *et al.* 1996) data.

Our principal finding, that presumed transisthmian pairs are often older than three million years, is likely to be true for many marine groups. Evidence for this comes from both other molecular analyses and the fossil record. Transisthmian divergence times for three genera of snails range from 5.3–8.5 Ma, based on an independent (non-Isthmian) calibration by means of the fossil record of a fourth genus (Collins 1989; T. M. Collins *et al.* 1996); these dates are entirely consistent with the pattern for *Alpheus* (figure 2). Four genera of echinoderms yield COI divergences of 4.7%, 9.8%, 12% and 17.6% (Hart *et al.* 1997; Metz *et al.* 1998; Lessios 1998); this span of divergences is remarkably similar to that observed for *Alpheus* (4–19% with a two-parameter Kimura correction), although the taxonomic range, gene regions, and corrections for these studies are not identical. Data for fish show even greater variability, with COI transisthmian divergences ranging over 60-fold (from 0.18–12.4%) across 17 genera (Bermingham *et al.* 1997). If we turn to the fossil record, most neotropical lineages of strombinid gastropod were restricted to either the Caribbean or eastern Pacific basins by 5 Ma (Jackson *et al.* 1993). L. S. Collins *et al.* (1996) argue that palaeoceanographic circulation models, isotopic evidence and foraminiferan originations all point to divergence of the basins beginning as early as 7–8 Ma.

These results have important implications for using the Isthmus to 'calibrate' molecular 'clocks'. Worries about the noisiness of molecular data have encouraged authors to focus on average values, particularly when they are consistent with past estimates. For example, two fish genera (*Abudefduf* and *Anisotremus*) each have two transisthmian pairs: in both genera, one pair has a divergence of 1.5% and the other 4.5% (Bermingham *et al.* 1997). In the context of the wide variation exhibited across all fish taxa and rate estimates from other studies, the 4.5% divergences were chosen as the best overall value for calibrations (Bermingham *et al.* 1997). It seems more likely, however, that (as with shrimp) the smaller divergences give better estimates based on the date of final closure, particularly when they have phylogenetic support (as is the case for *Abudefduf*, where only the pair with 1.5% divergence are sister species). This would imply a rate of change for this portion of the COI gene almost three times faster for shrimp than for fish (1.4% and 0.5% per million years, respectively).

In general, the shrimp data suggest that it is easy to overestimate rates of molecular evolution by failing to sample the appropriate transisthmian pairs. Taxa restricted to offshore, reef environments are likely to provide especially poor estimates of rates of molecular evolution, because divergence is likely to have preceded the closure of the Isthmus by an unknown amount of time. The best taxa for such estimates are mangrove associates, species routinely found in turbid, inshore waters, or high intertidal and quaterrestrial marine organisms (see, for example, Sturmbauer *et al.* 1996; Schubart *et al.* 1998). COI divergence values for the transisthmian estuarine crabs analysed by Schubart *et al.* (1988; 0.04, 0.06) were remarkably similar to those obtained for two estuarine *Alpheus* species (figure 2). Similarly, in the two fish genera that each have two transisthmian pairs, the lower divergences within each genus were found for pairs typically found on more inshore reefs (Allen & Robertson 1994). Even ecologically promising transisthmian taxa may individually yield unexpectedly high divergence values, however; although the mangrove-associated snails analysed by Collins (1989) had the lowest divergence values of the three pairs analysed, they still had values that suggested isolation before the close of the Isthmus based on rate calibrations for *Nucella* (Cunningham & Collins 1994).

Good estimates are important because temporal information allows one to associate cladogenesis with the physical history of the Earth and estimate the speed with which major evolutionary transitions take place. Our data suggest that some evolutionary transitions are likely to be considerably slower than several past transisthmian-based estimates would suggest. For example, Hart *et al.* (1997) used a transisthmian calibration for *Oreaster* (17.6%, three million years) to suggest that two major developmental changes in starfish may have occurred within the past two million years. However, as Hart *et al.* (1997) noted, if the calibration is incorrect (perhaps by over threefold, based on other echinoderm data), then these rapid evolutionary transitions are in fact considerably slower. Similarly, dates estimated for reconstructions of the biogeographic history of Central American freshwater fish (Bermingham *et al.* 1997) are too young if the

true rate of sequence divergence is 0.5% rather than 1.2%, the latter figure being derived from pairs that may have diverged well before closure of the Isthmus.

Finally, studies of transisthmian taxa support the idea that isolation in the sea can often occur without impermeable physical barriers (Palumbi 1994; Miya & Nishida 1997; Hellberg 1998). Where partial barriers do exist, biological responses to them are likely to be complex and drawn out in time, on land as well as sea, leading to phylogeographic patterns termed 'pseudocongruence' (Collins & Cunningham 1994). For example, divergence dates for North American birds traditionally attributed to the Late Pleistocene appear to range from 200 000 to over five million years based on mtDNA sequences (Klicka & Zink 1997). Similar findings of non-simultaneous divergences have also been reported across barriers in Australian and Brazilian rain forests (Joseph *et al.* 1995; Patton & da Silva 1998) and for terrestrial taxa separated by the opening of the Strait of Gibraltar (Busack 1986). Thus lessons learned from the Isthmus have broad applicability.

We thank Javier Jara and Adam Gerstein for assistance in the field, and Eyda Gomez for assistance in the laboratory. Steve Palumbi, Andrew Martin, Jeremy Jackson, Haris Lessios and an anonymous reviewer made helpful comments on earlier versions of this manuscript. Weibe Kooistra and Andrew Martin helped with analysis of rate constancy. The government of Panama (Recursos Marinos) and the Kuna Nation gave permission for fieldwork and collections, and the Smithsonian Institution and Field Museum provided financial support. This work is based in part upon data collected on a sequencer purchased with support from the US National Science Foundation (BIR-9419732) located in the Field Museum's Biochemistry Laboratories, renovated with support from the NSF (STI-921446) and operated with support from an endowment by the Pritzker Foundation.

REFERENCES

- Allen, G. R. & Robertson, D. R. 1994 *Fishes of the tropical eastern Pacific*. Bathhurst, UK: Crawford House Press.
- Bermingham, E. & Lessios, H. A. 1993 Rate variation of protein and mitochondrial DNA evolution as revealed by sea urchins separated by the Isthmus of Panama. *Proc. Natn. Acad. Sci. USA* **90**, 2734–2738.
- Bermingham, E., McCafferty, S. S. & Martin, A. P. 1997 Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. In *Molecular systematics of fishes* (ed. T. D. Kocher & C. A. Stepien), pp. 113–128. San Diego: Academic Press.
- Burton, R. S. & Lee, B.-N. 1994 Nuclear and mitochondrial gene genealogies and allozyme polymorphism across a major phylogeographic break in the copepod *Tigriopus californicus*. *Proc. Natn. Acad. Sci. USA* **91**, 5197–5201.
- Busack, S. D. 1986 Biogeographic analysis of the herpetofauna separated by the formation of the Strait of Gibraltar. *Natl Geogr. Res.* **2**, 17–36.
- Chace, F. A. Jr 1972 The shrimps of the Smithsonian-Bredin Caribbean expeditions with a summary of the West Indian shallow-water species (Crustacea: Decapoda: Natantia). *Smithson. Contrib. Zool.* **98**, 1–179.
- Chenoweth, S. F., Hughes, J. M., Keenan, C. P. & Lavery, S. 1998 When oceans meet: a teleost shows secondary intergradation at an Indian–Pacific interface. *Proc. R. Soc. Lond. B* **265**, 415–420.
- Coates, A. G. & Obando, J. A. 1996 The geological evolution of the Central American Isthmus. In *Evolution and environment in tropical America* (ed. J. B. C. Jackson, A. F. Budd & A. G. Coates), pp. 21–56. University of Chicago Press.
- Collins, L. S., Budd, A. F. & Coates, A. G. 1996 Earliest evolution associated with closure of the Tropical American Seaway. *Proc. Natn. Acad. Sci. USA* **93**, 6069–6072.
- Collins, T. M. 1989 *Rates of mitochondrial DNA evolution in transisthmian geminate species*. PhD dissertation, Yale University, New Haven, Connecticut.
- Collins, T. 1996 Molecular comparisons of transisthmian species pairs: rates and patterns of evolution. In *Evolution and environment in tropical America* (ed. J. B. C. Jackson, A. F. Budd & A. G. Coates), pp. 303–334. University of Chicago Press.
- Collins, T. M., Frazer, K., Palmer, A. R., Vermeij, G. J. & Brown, W. M. 1996 Evolutionary history of northern hemisphere *Nucella* (Gastropoda, Muricidae): molecular, morphological, ecological, and paleontological evidence. *Evolution* **50**, 2287–2304.
- Cronin, T. M. & Dowsett, H. J. 1996 Biotic and oceanographic response to the Pliocene closing of the Central American Isthmus. In *Evolution and environment in tropical America* (ed. J. B. C. Jackson, A. F. Budd & A. G. Coates), pp. 76–104. University of Chicago Press.
- Cunningham, C. W. & Collins, T. M. 1994 Developing model systems for molecular biogeography: vicariance and inter-change in marine invertebrates. In *Molecular ecology and evolution: approaches and applications* (ed. B. Schierwater, B. Streit, G. P. Wagner & R. DeSalle), pp. 405–433. Basle, Switzerland: Birkhäuser.
- Cunningham, C. W. & Collins, T. M. 1998 Beyond area relationships: extinction and recolonization in molecular marine biogeography. In *Molecular approaches to ecology and evolution* (ed. R. DeSalle & B. Schierwater). Basle, Switzerland: Birkhäuser. (In the press.)
- Farrell, J. W., Raffi, I., Janecek, T. R., Murray, D. W., Levitan, M., Dadey, K. A., Emeis, K.-C., Lyle, M., Flores, J.-A. & Hovan, S. 1995 Late Neogene sedimentation patterns in the eastern equatorial Pacific Ocean. *Proc. Ocean Drill. Prog., Scient. Res.* **138**, 717–756.
- Goldman, N. 1994 Variance to mean ratio, $R(t)$, for Poisson processes on phylogenetic trees. *Molec. Phylog. Evol.* **3**, 230–239.
- Grosberg, R. K. 1982 Intertidal zonation of barnacles: the influence of planktonic zonation of larvae on vertical distribution of adults. *Ecology* **63**, 894–899.
- Hart, M. W., Byrne, M. & Smith, M. J. 1997 Molecular phylogenetic analysis of life-history evolution in asterinid starfish. *Evolution* **51**, 1848–1861.
- Hellberg, M. E. 1998 Sympatric sea shells along the sea's shore: the geography of speciation in the marine gastropod *Tegula*. *Evolution*. (In the press.)
- Jackson, J. B. C., Jung, P., Coates, A. G. & Collins, L. S. 1993 Diversity and extinction of tropical American mollusks and emergence of the Isthmus of Panama. *Science* **260**, 1624–1626.
- Jackson, J. B. C., Jung, P. & Fortunato, H. 1996 Paciphillia revisited: transisthmian evolution of the *Strombina* group (Gastropoda: Columbellidae). In *Evolution and environment in tropical America* (ed. J. B. C. Jackson, A. F. Budd & A. G. Coates), pp. 234–270. University of Chicago Press.
- Joseph, L., Moritz, C. & Hugall, A. 1995 Molecular support for vicariance as a source of diversity in rainforest. *Proc. R. Soc. Lond. B* **260**, 177–182.
- Kim, W. & Abele, L. G. 1988 The snapping shrimp genus *Alpheus* from the eastern Pacific (Decapoda: Caridea: Alpheidae). *Smithson. Contrib. Zool.* **454**, 1–119.
- Klicka, J. & Zink, R. M. 1997 The importance of recent ice ages in speciation: a failed paradigm. *Science* **277**, 1666–1669.

- Knowlton, N. & Mills, D. E. K. 1992 The systematic importance of color and color pattern: evidence for complexes of sibling species of snapping shrimp (Caridea: Alpheidae: *Alpheus*) from the Caribbean and Pacific coasts of Panama. *Proc. San Diego Soc. Nat. Hist.* **18**, 1–5.
- Knowlton, N. & Weigt, L. A. 1997 Species of marine invertebrates: a comparison of the biological and phylogenetic species concepts. In *Species: the units of biodiversity* (ed. M. F. Claridge, H. A. Dawah & M. R. Wilson), pp. 199–219. London: Chapman & Hall.
- Knowlton, N., Weigt, L. A., Solórzano, L. A., Mills, D. K. & Bermingham, E. 1993 Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. *Science* **260**, 1629–1632.
- Lessios, H. A. 1979 Use of Panamanian sea urchins to test the molecular clock. *Nature* **280**, 599–601.
- Lessios, H. A. 1998 The first stage of speciation as seen in organisms separated by the Isthmus of Panama. In *Endless forms: species and speciation* (ed. D. Howard & S. Berlocher). Oxford University Press. (In the press.)
- McMillan, W. O. & Palumbi, S. R. 1995 Concordant evolutionary patterns among Indo-west Pacific butterflyfishes. *Proc. R. Soc. Lond. B* **260**, 229–236.
- Martin, A. P., Naylor, G. J. P. & Palumbi, S. R. 1992 Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. *Nature* **357**, 153–155.
- Metz, E. C., Gómez-Gutiérrez, G. & Vacquier, V. D. 1998 Mitochondrial DNA and bindin gene sequence evolution among allopatric species of the sea urchin genus *Arbacia*. *Molec. Biol. Evol.* **15**, 185–195.
- Miya, M. & Nishida, M. 1997 Speciation in the open sea. *Nature* **389**, 803–804.
- Morrison, C. L. 1997 *Patterns of nucleotide substitution and lineage-specific rates of divergence among snapping shrimp separated by the Isthmus of Panama*. PhD dissertation, Florida State University, Tallahassee, Florida.
- Palumbi, S. R. 1994 Genetic divergence, reproductive isolation, and marine speciation. *A. Rev. Ecol. Syst.* **25**, 547–572.
- Palumbi, S. R. 1996 What can molecular genetics contribute to marine biogeography? An urchin's tale. *J. Exp. Mar. Biol. Ecol.* **203**, 75–92.
- Patton, J. L. & da Silva, M. N. F. 1998 Rivers, refuges, and ridges: the geography of speciation of Amazonian mammals. In *Endless forms: species and speciation* (ed. D. Howard & S. Berlocher). Oxford University Press. (In the press.)
- Schubart, C. D., Diesel, R. & Hedges, S. B. 1998 Rapid evolution to terrestrial life in Jamaican crabs. *Nature* **393**, 363–365.
- Strimmer, K., Goldman, N. & van Haeseler, A. 1997 Bayesian probabilities and quartet puzzling. *Molec. Biol. Evol.* **14**, 210–211.
- Sturmbauer, C., Levinton, J. S. & Christy, J. 1996 Molecular phylogeny analysis of fiddler crabs: test of the hypothesis of increasing behavioral complexity in evolution. *Proc. Natn. Acad. Sci. USA* **93**, 10 855–10 857.
- Vawter, A. T., Rosenblatt, R. & Gorman, G. C. 1980 Genetic divergence among fishes of the eastern Pacific and the Caribbean: support for the molecular clock. *Evolution* **34**, 705–711.
- Vermeij, G. J. 1991 When biotas meet: understanding biotic interchange. *Science* **253**, 1099–1104.
- Wu, C.-I. & Li, W.-H. 1985 Evidence for higher rates of nucleotide substitution in rodents than in man. *Proc. Natn. Acad. Sci. USA* **82**, 1741–1745.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.

