

UNDERSTANDING THE ORIGINS OF AREAS OF ENDEMISM IN PHYLOGEOGRAPHIC ANALYSES: A REPLY TO BRIDLE ET AL.

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One of the chief strategies used to protect biodiversity is the identification of areas of endemism—regions in which unique species or populations are concentrated. It often has been assumed that such areas result from nonrandom historical processes that affect a biota, such as vicariance of distributional ranges by geological or climatological events. This might produce concordant patterns of distribution, as well as genetic divergence, among clades of distantly related species, such as monkeys, toads, and grasshoppers. However, explanations due to vicariance and fragmentation have appropriately come under closer scrutiny, because independent processes, such as adaptation to local ecological conditions or lineage sorting, might produce similar biogeographic patterns. Hence the use of hypothesis testing in biogeography, as opposed to narrative scenarios, has grown (Knowles 2001; Templeton 2002; Lessa et al. 2003).

We recently identified concordant areas of endemism (AOEs) in monkeys and toads on the biodiverse Indonesian island of Sulawesi that we attributed to habitat fragmentation (Evans et al. 2003a). Bridle et al. (2004) proposed that the identification of these AOEs is premature. They presented an alternative analysis and concluded that a hypothesis of isolation by distance would explain the distributional patterns in the Celebes toad, *Bufo celebensis*. Thus, the main concerns of Bridle et al. (2004) are: (1) other processes in addition to or instead of fragmentation, such as isolation by distance, may explain the distribution of diversity on Sulawesi, (2) not all Sulawesi species or populations have AOEs that correspond with Sulawesi macaques and toads, and (3) a hypothesis of isolation by distance rather than fragmentation is sufficient to explain our data from *B. celebensis* mitochondrial DNA (mtDNA). We agree with the first two of these assertions, as we have discussed (Evans et al. 2003a), but disagree with the third.

In this response we review evidence that supports fragmentation as the cause of concordant AOEs in Sulawesi monkeys and toads. We describe how assumptions made in the partial correlation test by Bridle et al. (2004) are not satisfied by the data, and how these inappropriate assumptions deliver a misleading result. We further demonstrate that when these assumptions are not made, our data on *B. celebensis* strongly support a significant correlation between genetic distance and Sulawesi AOEs that is independent of geographical distance, a result that is also supported by our other analyses (Evans et al. 2003a). Finally, we point out that although exceptions to a more common pattern of endemism are expected, these

areas provide useful biological information for conservation decisions.

Null hypotheses, nested clade analysis

Evans et al. (2003a) tested the null hypothesis of panmixia of *B. celebensis* mtDNA with nested clade analysis (NCA); deviations from the null were explored with alternative explanations including isolation by distance and fragmentation (Templeton 1998). Bridle et al. (2004) criticized the use of NCA, and cited a critique of NCA that we also cited by Knowles and Maddison (2002). However, they did not demonstrate how NCA might have caused error in our study. One conclusion of Knowles and Maddison (2002) is that NCA has a tendency to assign deterministic processes such as fragmentation to patterns that may be realized by stochastic processes such as lineage sorting. Although this seems plausible for a small number of congruent patterns, the “mirror image” of seven separate AOEs for toads and monkeys is supported by phylogenetic analyses and by parametric bootstrap tests, and argues against stochastic lineage sorting as the sole cause of the phylogeographic pattern of *B. celebensis* mtDNA.

If abiotic processes influenced the distribution of diversity on Sulawesi, multiple sympatrically occurring species with different ecological adaptations might share a common distribution of diversity. Bridle et al. (2004) suggested that we incorrectly tested the null hypothesis that they embrace—that genetic variation is due to isolation by distance (a hypothesis we tested with NCA). However, they missed the point that the null hypotheses of the parametric bootstrap tests (Huelssenbeck et al. 1996; Goldman et al. 2000) explored another alternative: that *B. celebensis* mtDNA sequences form a clade in each AOE.

Isolation by distance is expected to result in older lineages typically being widespread, whereas younger ones have a more limited distribution (Crandall and Templeton 1993; Posada and Crandall 2001). Although stochastic processes or poor sampling can yield no overlap without any barrier to gene flow (Irwin 2002), in the absence of barriers to dispersal other than distance some geographic overlap in clades can result. That we detected no geographic overlap of the two oldest mtDNA clades of *B. celebensis* and also no overlap of seven other divergent clades suggests the role of factors in addition to geographic distance in influencing AOEs. Of course, as Bridle et al. (2004) pointed out, multiple molecular markers and other sources of information are preferable to a

single genealogy. However, our study tested for congruence of the AOEes of toads on Sulawesi with another taxon, the macaques. Congruence of AOEes of multiple species suggests a common mechanism (Avice 2000) in the same way that congruence among multiple molecular markers does in one species.

Moreover, a recent study of fanged frogs (*Limnonectes*) found almost identical AOEes, further corroborating the non-stochastic nature of the multitaxon congruence (Evans et al. 2003b). Three sympatric species complexes occur in the North Central and Northeast AOEes. Each complex has a clade of mtDNA that may be restricted to the North Central AOE and another clade that may be restricted to the Northeast AOE. Three other species of fanged frog occur exclusively in the Southwest AOE and two species occur exclusively in the West Central AOE. Two species also have over 95% of their distributions restricted to the Northwest AOE.

Partial correlation between genetic distance and AOEes

Bridle et al. (2004) used partial correlation tests to control for the effect of geographic distance on the correlation between evolutionary divergence and AOEes in *B. celebensis* mtDNA. The null hypothesis of this test is that the genetic distances among individuals within AOEes are not different from those among individuals from separate AOEes, once the effect of geographic distance is held constant. The hypothesis of AOEes predicts an alternative: that genetic distances are lower among individuals within AOEes than among individuals from separate AOEes, once the effect of geographic distance is held constant. Put another way, the AOE hypothesis predicts that individuals within an AOE have closer evolutionary relationships than individuals in different AOEes.

A basic assumption is that genetic distances provide an estimate of the evolutionary divergence among the terminals. However, Bridle et al. (2004) used pairwise genetic distances for their partial correlation test that were not calculated in the context of a phylogeny, and that do not accurately reflect evolutionary relationships among the sequences as estimated by phylogenetic analyses. There is an imperfect fit between the true genealogy and pairwise genetic distances because these distances are not additive (Felsenstein 1988). Furthermore, when evolution is not clocklike, pairwise genetic distances can misrepresent the evolutionary distance of DNA sequences (Felsenstein 1984; Rzhetsky and Sitnikova 1996). In the present case, a likelihood ratio test rejects the hypothesis of clocklike evolution of *B. celebensis* mtDNA sequences, either with outgroup sequences ($P < 0.0001$, $df = 18$) or without them ($P < 0.0001$, $df = 14$). As a result, some of the pairwise distances of Bridle et al. (2004), for example, are smaller between nonsister sequences than between sister sequences (Fig. 1). Thus the amount of divergence within AOEes is exaggerated, and the amount of divergence across AOEes is underestimated. These issues cast doubt on the result of their partial correlation test which finds no significant correlation between pairwise genetic distances and AOEes while holding geographic distance constant ($r = 0.0725$; $P = 0.086$; Bridle et al. 2004).

We addressed these issues in two ways. First we constructed a matrix of patristic genetic distances along branches

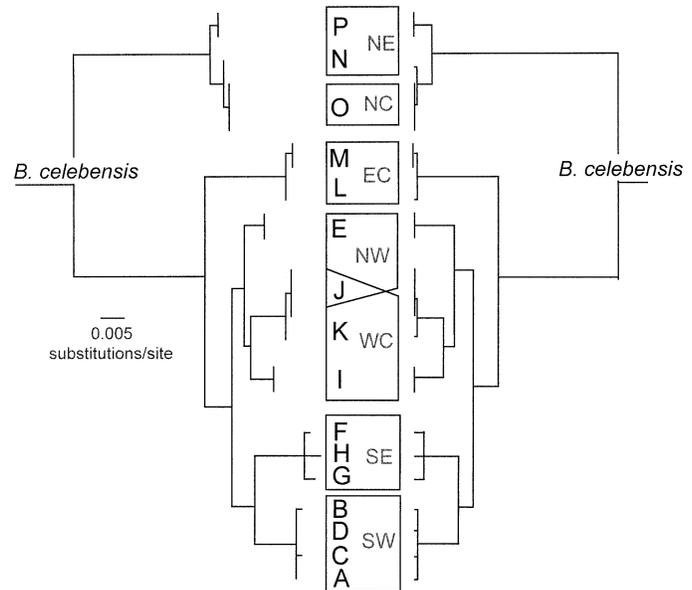


Fig. 1. The partial correlation test of Bridle et al. (2004) used nonadditive pairwise genetic distances that warp evolutionary relationships, especially when evolution is not clocklike. For example, the pairwise genetic distance between sequences E and J (0.02815), used by Bridle et al. (2004), is larger than their pairwise distance for the evolutionarily more distant comparison between E and C (0.02374). Their pairwise genetic distance between sequences J and I (0.02284) is larger than that for the evolutionarily more distant comparison between sequences E and I (0.01779). On the left is a rooted maximum likelihood (ML) tree estimated from unique *B. celebensis* mtDNA sequences. On the right is a chronogram constructed from the ML topology allowing for variable rates in evolution on different branches. The topology of these trees is identical to those obtained from Bayesian and maximum parsimony analysis in Evans et al. (2003a); the ML tree was estimated using the same model (TrN + Γ) and same parameter settings as the pairwise genetic distances used by Bridle et al. (2004). Bold capital letters refer to unique sequences and are boxed and labeled according to Sulawesi AOEes, as in Evans et al. (2003a).

of a maximum likelihood tree (Fig. 1). Patristic distances represent the total length of branches between each pair of sequences in the tree (Farris 1967) and are additive. Patristic genetic distances tend to be smaller between closely related sequences than between more distantly related sequences, although this is not necessarily the case because of homoplasy and uneven rates of evolution. For example, the patristic distance on the maximum likelihood tree between sequences J and I is still slightly larger than the patristic distance between the more distantly related sequences E and I (see Supplementary Information, available online at <http://dx.doi.org/10.1554/CR04-001.1.s1>). Reanalysis using the partial correlation test with patristic genetic distances yields a highly significant correlation between genetic distance and AOEes while holding geographic distance constant ($r = 0.240981$, $P = 0.00005$), a result that is consistent with the AOE hypothesis.

Second, to adjust for uneven rates of evolution, we used the r8s program version 1.60 (Sanderson 1997, 2002) to construct a chronogram from the maximum likelihood topology (Fig. 1). This program allows for unequal rates of evolution on different branches but uses a smoothing parameter to min-

imize the disparity among rates from branch to branch. Because branch lengths of a chronogram are proportional to time, patristic distances along a chronogram are additive and also accurately reflect the genealogical hierarchy among the sequences: the genetic distance between closely related sequences necessarily is smaller than the distance between more distantly related sequences. A cross-validation procedure selected an appropriate smoothing parameter for r8s, and the most recent common ancestor of all *B. celebensis* sequences was set to an arbitrary age of one. Using patristic genetic distances from the chronogram, the partial correlation test again recovered a highly significant correlation between genetic distance and AOE, while holding geographic distance constant ($r = 0.222878$, $P = 0.00019$). We conclude that geographic distance alone cannot account for the pattern of molecular variation we observe in *B. celebensis*.

Congruence and incongruence of AOE

We did not observe exact congruence in all contact zones of monkeys and toads on a minute geographic scale, nor do we expect it in other organisms. A lack of precise matching of contact zones is not sufficient evidence for abandoning a hypothesis of fragmentation on Sulawesi because the barriers that originally caused fragmentation are probably no longer present. Evans et al. (2003a) discussed how fine scale differences in AOE are attributable to variation in dispersal, demography, ancestral population structure, and selection on different species and populations. Contact zones potentially can change in width or move considerable distances (Dasmahapatra et al. 2002) when they are not maintained by a stable environmental gradient or physical barrier (Barton and Hewitt 1985). However, because these barriers retreated recently (Hall 2001; Whitten et al. 2002) substantial overlap of areas of endemism is expected in many groups. Indeed, intraspecific mtDNA clades of Sulawesi *Chitaura* grasshoppers, for example, are almost entirely restricted to one of the AOE as defined by monkeys and toads (Walton et al. 1997; Butlin et al. 1998). *Chitaura* color morphs are also generally restricted to one AOE (Bridle et al. 2001), although the species status of these morphs is unclear (R. Butlin, pers. comm.). For some organisms, additional AOE may also exist inside the general ones of monkeys and toads. In the West Central AOE, for example, flying lizards (*Draco*) have highly differentiated mtDNA (J. McGuire, pers. comm.) and aquatic true bugs (Heteroptera) have separate AOE (Polhemus and Polhemus 1990).

Examples of incongruent distributions, however, undoubtedly are present in some species. A fanged frog, "*Limnonectes* sp., I" for example, has intraspecific mtDNA clades that are not consistent with general AOE of Sulawesi monkeys and toads (Evans et al. 2003b). This species reached Sulawesi separately from other fanged frogs, and its ancestor also successfully dispersed to the Moluccas over permanent marine barriers (Evans et al. 2003b). Areas of endemism of plants with wind dispersal may also, for example, be quite different from those of many terrestrial animals. However, if we ignore patterns that are shared by many (but not all) organisms, conservation decisions will have little biological basis.

Conservation

One goal of biodiversity conservation is the sustainable preservation of genetic variation within species. On Sulawesi, biodiversity is not evenly distributed—in many species it is clustered in pockets, or areas of endemism, that correspond with the distributions of the Sulawesi macaque species. At the moment, some of these, such as the Southwest, Northwest, and East Central AOE, are virtually unprotected and in dire need of conservation action. Today the principal environmental transition in Indonesia is deforestation (Jepson et al. 2001), and delay in implementation of a regional approach to target Sulawesi AOE will result in substantial loss of biodiversity on this unique island.

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LITERATURE CITED

- Avice, J. C. 2000. Phylogeography. The history and formation of species. Harvard Univ. Press, Cambridge, MA.
- Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. *Annu. Rev. Ecol. Syst.* 16:113–148.
- Butlin, R. K., C. Walton, K. A. Monk, and J. R. Bridle. 1998. Biogeography of Sulawesi grasshoppers, genus *Chitaura*, using DNA sequence data. Pp. 355–359 in R. Hall and D. J. Holloway, eds. *Biogeography and geological evolution of Southeast Asia*. Backhuys Publishers, Leiden, The Netherlands.
- Bridle, J. R., A. Garn, K. A. Monk, and R. K. Butlin. 2001. Speciation in *Chitaura* grasshoppers (Acrididae: Oxyinae) on the island of Sulawesi: colour patterns, morphology and contact zones. *Biol. J. Linn. Soc.* 72:373–390.
- Bridle, J. R., P. M. Pedro, and R. K. Butlin. 2004. Habitat fragmentation and biodiversity: testing for the evolutionary effects of refugia. *Evolution* 58:1394–1396.
- Crandall, K. A., and A. R. Templeton. 1993. Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* 134:959–969.
- Dasmahapatra, K. K., M. J. Blum, A. Aiello, S. Hackwell, N. Davies, E. P. Bermingham, and J. Mallet. 2002. Inferences from a rapidly moving hybrid zone. *Evolution* 56:741–753.
- Evans, B. J., J. Supriatna, N. Andayani, M. I. Setiadi, D. C. Cannatella, and D. J. Melnick. 2003a. Monkeys and toads define areas of endemism on Sulawesi. *Evolution* 57:1436–1443.
- Evans, B. J., R. M. Brown, J. A. McGuire, J. Supriatna, N. Andayani, A. Diesmos, D. T. Iskandar, D. J. Melnick, and D. C. Cannatella. 2003b. Phylogenetics of fanged frogs (Anura; Ranidae; *Limnonectes*): testing biogeographical hypotheses at the Asian-Australian faunal zone interface. *Syst. Biol.* 52:794–819.
- Farris, J. S. 1967. The meaning of relationship and taxonomic procedure. *Syst. Zool.* 16:44–51.
- Felsenstein, J. 1984. Distance methods for inferring phylogenies: a justification. *Evolution* 38:16–24.
- . 1988. Phylogenies from molecular sequences: inference and reliability. *Annu. Rev. Genet.* 22:521–565.
- Goldman, N., J. P. Anderson, and A. G. Rodrigo. 2000. Likelihood-based tests of topologies in phylogenetics. *Syst. Biol.* 49:652–670.
- Hall, R. 2001. Cenozoic reconstructions of SE Asia and the SW Pacific: changing patterns of land and sea. Pp. 35–56 in I. Metcalfe, J. Smith, M. Morwood, and I. Davidson, eds. *Faunal and floral migrations and evolution in SE Asia-Australia*. Swets and Zeitlinger Publishers, Lisse, Switzerland.
- Huelsenbeck, J. P., D. M. Hillis, and R. Jones. 1996. Parametric bootstrapping in molecular phylogenetics: applications and performance. Pp. 19–45 in J. D. Ferraris and S. R. Palumbi, eds.

- Molecular zoology: advances, strategies, and protocols. Wiley-Liss, New York.
- Irwin, D. E. 2002. Phylogeographic breaks without geographic barriers to gene flow. *Evolution* 56:2383–2394.
- Jepson, P., J. K. Jarvie, L. MacKinnon, and K. A. Monk. 2001. The end of Indonesia's lowland forests? *Science* 292:859–861.
- Knowles, L. L. 2001. Genealogical portraits of speciation in montane grasshoppers (genus *Melanoplus*) from the sky islands of the Rocky Mountains. *Proc. R. Soc. Lond.* 268:319–324.
- Knowles, L. L., and W. P. Maddison. 2002. Statistical phylogeography. *Mol. Ecol.* 11:2623–2635.
- Lessa, É. P., J. A. Cook, and J. L. Patton. 2003. Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. *Proc. Natl. Acad. Sci. USA* 100:10331–10334.
- Polhemus, J. T., and D. A. Polhemus. 1990. Zoogeography of the aquatic Heteroptera of Celebes: regional relationships versus insular endemism. Pp. 73–86 in W. J. Knight and J. D. Holloway, eds. *Insects and the rain forests of South East Asia*. The Royal Entomological Society of London, London.
- Posada, D., and K. A. Crandall. 2001. Intraspecific gene genealogies: trees grafting into networks. *Trends Ecol. Evol.* 16:37–45.
- Rzhetsky, A., and T. Sitnikova. 1996. When is it safe to use an oversimplified substitution model in tree-making? *Mol. Biol. Evol.* 13:1255–1265.
- Sanderson, M. J. 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Mol. Biol. Evol.* 19:101–109.
- . 2002. Estimating absolute rates of molecular evolution and divergence times in the absence of rate consistency. *Mol. Biol. Evol.* 19:1218–1231.
- Templeton, A. R. 1998. Nested clade analysis of phylogeographic data: testing hypotheses about gene flow and population history. *Mol. Ecol.* 7:381–397.
- . 2002. Out of Africa again and again. *Nature* 416:45–51.
- Walton, C., R. K. Butlin, and K. A. Monk. 1997. A phylogeny for grasshoppers of the genus *Chitaura* (Orthoptera: Acrididae) from Sulawesi, Indonesia, based on mitochondrial DNA sequence data. *Biol. J. Linn. Soc.* 62:365–382.
- Whitten, T., G. S. Henderson, and M. Mustafa. 2002. *The ecology of Sulawesi*. Periplus Editions Ltd., Hong Kong.

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