Phylogenetic trees support the coevolution of parasites and their hosts

Mark S. Hafner & Steven A. Nadler

Museum of Zoology and Department of Zoology and Physiology, Louisiana State University, Baton Rouge, Louisiana 70803, USA

The close correspondence often observed between the taxonomy of parasites and their hosts[1,2] has led to Fahrenholz's rule[3], which postulates that parasites and their hosts speciate in synchrony. This leads to the prediction that phylogenetic trees of parasites and their hosts should be topologically identical[2,4]. We report here a test of this prediction which involves the construction of phylogenetic trees for rodents and their ectoparasites using protein electrophoretic data. We find a high degree of concordance in the branching patterns of the trees which suggests that there is a history of cospeciation in this host–parasite assemblage. In several cases where the branching patterns were identical in the host and parasite phylogenies, the branch lengths were also very similar which, given the assumptions of molecular clock theory, strongly suggests that the speciation of these hosts and ectoparasites was roughly contemporaneous and causally related.

We have performed a direct test of Fahrenholz's rule by comparing independent phylogenies based on biochemical data for a group of rodents and their ectoparasites. The rodents in this study include eight species of pocket gophers, representing three genera in the rodent family Geomyidae; these fossorial herbivores are usually solitary, and geomyid species are generally allopatric. The ectoparasites include ten species of chewing lice representing two genera in the mallophagan family Trichodectidae. The life cycle of these wingless insects occurs entirely on the host and includes three principal stages: egg, nymph, and adult. Chewing lice have a generation time of ~40 days[5], which is roughly one-fifth the generation time of pocket gophers[6]. Transmission of chewing lice among pocket gophers is thought to occur only through host-to-host contact[7], and the combination of low parasite vagility and obligate contact-transmission of lice should limit opportunities for colonization of new host species. The absence of widespread transfer of lice among host species should, in turn, increase the likelihood of detecting cospeciation in this host–parasite assemblage.

Using standard electrophoretic procedures[8], we surveyed 31 protein loci in pocket gophers and 14 loci in chewing lice brushed from the pelage of the pocket gophers. To ensure that our electrophoretic survey of lice was not an examination of pocket gopher proteins contained in the gut of the louse, we compared protein variation at the ten loci in common to the two surveys. At all ten loci, hosts and parasites showed different electromorphs and different patterns of variation (Fig. 1). Each data set was clustered using both cladistic (locus-by-locus[9,10]) and phenetic[11] procedures; tree topology was unaffected by the clustering method used.

The host and parasite trees are topologically identical in all but three regions (indicated by daggers in Fig. 2). In most cases, sister taxa of lice parasitize hosts that are also sister taxa (see nodes A and F, Fig. 2), and branching sequences above the species level in the two groups are identical (nodes B–E). The probability of this level of topological similarity occurring by chance alone was calculated using the component-replication method of Nelson and Platnick[12]. This method requires equal numbers of taxa in the trees being compared, making it necessary for us to reduce the number of taxa in our louse tree. Because each species of *Thomomys* hosts two species of lice (Fig. 2), we first calculated probability levels excluding the two *Thomomydoecus* species, *wardi* and *minor*. The probability of chance similarity of the two trees (without *wardi* and *minor*)
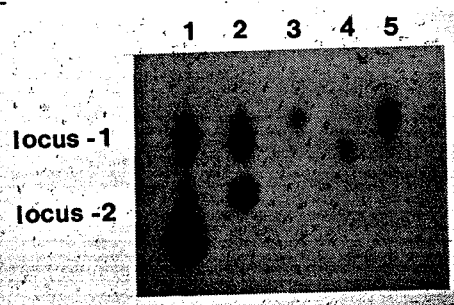

Fig. 1 An example of electrophoretic variation at one of ten protein loci (isocitrate dehydrogenase-1) examined in both pocket gophers and lice. Pocket gopher samples are in lanes 1 and 2, lice in lanes 3–5. Allele designations at locus 1 are listed after species names: lane 1, *Orthogeomys heterodus* A; lane 2, *Thomomys bottae* A; lane 3, *Thomomydoecus minor* b; lane 4, *Geomydoecus actuosi* d; lane 5, *Geomydoecus costaricensis* c. The pocket gopher in lane 1 hosts the louse species in lane 5, and the lice in lanes 3 and 4 coexist on the host in lane 2 (see Fig. 2). Only one isocitrate dehydrogenase locus was evident in lice.

was low (P = 0.05). However, when *wardi* and *minor* were included and the two *Geomydoecus* species (*thomomyus* and *actuosi*) excluded, the probability of chance similarity of the host and parasite trees was remote (P = 0.001). This high degree of tree matching is consistent with predictions of the cospeciation hypothesis.

The three host–parasite associations indicated by asterisks in Fig. 2 probably result from host switching (lateral transfer) by lice. In these cases, the phylogenetic history of the louse taxon does not mirror that of its host, thereby falsifying the cospeciation hypothesis for these (and only these) associations. It is important to note that no case of suspected host switching involves hosts that are geographically disjunct; in each case, the geographical range of the colonizer's current host abuts that of the colonizer's putative ancestral host (that is, the pocket gopher parasitized by the colonizer's sister taxon)[13].

Not only does the cospeciation hypothesis predict similar patterns of speciation in host–parasite assemblages, but it also predicts that the speciation events were causally related and therefore, approximately contemporaneous. Thus, we can test the cospeciation hypothesis from another perspective if we assume that proteins evolve in a roughly clock-like fashion. If hosts and parasites are actually speciating in synchrony then, according to the 'molecular clock' hypothesis[15,16], we should expect to see roughly equivalent amounts of protein change in associated host and parasite lineages following speciation events (represented by nodes on phylogenetic trees). Therefore, to be consistent with the strict cospeciation model, host and parasite phylogenies must agree not only in branching pattern, but also in branch lengths, which are proportional to amounts of protein change in Fig. 2. Estimates of genetic distance, hence branch lengths, may be influenced by the ratio of slow:fast evolving loci surveyed[17]; in this study, 4 of 31 loci (13%) examined in pocket gophers, and 2 of 14 loci (14%) surveyed in chewing lice, can be classified as fast-evolving[18].

Analogous nodes on the two trees (A–F in Fig. 2) represent hypothesized cospeciation events in the history of the assemblage. Four of these nodes (A, C, D, E) are positioned at very similar genetic distances in the two trees (≤4% different in each case), and we interpret this concordance as further corroboration of the cospeciation hypothesis. Specifically, we suggest that the speciation events represented by nodes A, C, and E were approximately contemporaneous, and that the host and parasite lineages involved have accumulated protein differences at equal rates.

The dissimilarity between host and parasite genetic distance at nodes F in Fig. 2 suggests that the speciation events represented by these nodes were not contemporaneous (in that speci-

LETTERS TO NATURE

Fig. 2 Phenograms of pocket gopher and louse relationships based on protein electrophoretic data and using Rogers genetic distance metric[19]. Host–parasite associations are indicated by lines connecting extant species, with asterisks indicating probable instances of host switching. Note that the louse *setzeri* is found on two host species, and certain hosts (*talpoides*, *bottae* and *cherriei*) harbour two species of lice; in the latter cases, the louse species are usually sympatric on an individual pocket gopher. Daggers identify nodes on the louse tree that are absent on the pocket gopher tree. Electrophoretic data were clustered using UPGMA (ref. 11). The cophenetic correlation coefficient is 0.98 for the *Orthogeomys* portion of the pocket gopher tree and 0.97 for the louse tree. Loci examined[8] in pocket gophers were: alcohol dehydrogenase, sorbitol dehydrogenase, lactate dehydrogenase-1, lactate dehydrogenase-2, malate dehydrogenase-1, malate dehydrogenase-2, malic enzyme, isocitrate dehydrogenase-1, isocitrate dehydrogenase-2, glucose dehydrogenase, glucose-6-phosphate dehydrogenase, alpha-glycerophosphate dehydrogenase, superoxide dismutase-1, superoxide dismutase-2, glutamate-oxaloacetate transaminase-1, glutamate-oxaloacetate transaminase-2, creatine kinase-1, creatine kinase-2, adenylate kinase, 4-methyl-umbelliferyl acetate esterase, peptidase A, peptidase B, peptidase C, leucine aminopeptidase, fumarate hydratase, aconitase-1, aconitase-2, mannose phosphate isomerase, glucose phosphate isomerase, albumin and haemoglobin. Allele designations at each locus (with loci in the sequence listed above) for the eight pocket gopher species are as follows: *Thomomys talpoides*: C, C, C, A, A, D, D, A, E, D, E, E, C, D, E, A, A, E, E, E, B, H, C, D, C, B, G, E, C, B, D, A; *T. bottae*: D, D, C, A, A, D, D, A, D, C, D, D, C, D, D, A, D, D, D, B, G, B, D, B, C, F, D, B, C, C, A; *Geomys bursarius*: E, A, B, A, A, C, C, A, C, B, C, C, B, C, A, A, C, C, C, A, F, A, C, A, B, E, C, A, A, A, A; *Orthogeomys hispidus*: B, A, B, A, A, B, B, A, B, B, A, B, A, E, A, B, A, A, A, B, A, A, B, A; *O. cavator*: A, B, B, A, A, A, A, A, A, A, B, A, A, A, B, A, A, A, A, A, D, A, A, A, A, D, A, A, A, A, A; *O. underwoodi*: A, A, B, A, A, A, A, A, A, A/B, A, A, A, A, A, A, A, A, A, C, A, A, A, A, C, A, A, A, A, A; *O. cherriei*: A, A, A/B, A, A, A, A, A, A, A, A/B, A, A, A, A/B, A, A, A, A, A, A; *O. heterodus*: A, A, A/B, A, A, A, A, A, A, A, B, A, A, A, A, A, A, A, A, A, A, A, A, A, A, A, A, A/B, A, A, A, A. Loci examined in chewing lice were: malate dehydrogenase, malic enzyme, isocitrate dehydrogenase-1, xanthine dehydrogenase, superoxide dismutase-1, superoxide dismutase-2, arginine kinase, 4-methyl-umbelliferyl acetate esterase, alpha-naphthyl acetate esterase, peptidase A, peptidase C, adenosine deaminase, fumarate hydratase and glucose phosphate isomerase. Allele designations at each locus (with loci in sequence listed above) for the ten louse species are as follows: *Thomomydoecus wardi*: a, c, b, h, d, b, b, d, d/f, a, d, b, a, h; *T. minor*: a, c, b, g, e, b, b, d, d/f, a, d, b, a, i; *Geomydoecus thomomyus*: a, b, a, f, b, a, b, c, d/e, a, a, c, a, e; *G. actuosi*: a, b, d, e, a, a, b, c, d/f/g, a, a, c, a, f/g; *G. ewingi*: a, b, d, d, a, a, b, c, d/f, a, b, c, a, f; *G. yucatanensis*: a, a, c, b, c, a, a, c, c/d, b, e, d, a, c; *G. panamensis*: a, b, c, c, c, a, a, b, b/c, a, d, a, a, d; *G. setzeri*: a, b, c, c, c, a, a, b, a/b, a, c, a, a, a; *G. cherriei*: a, b, c, b, c, a, a, a, b, a, a, b, b; *G. costaricensis*: a, b, c, a, c, a, a, a, b/d, a, a, a, b, b.

Pocket gophers   Host–parasite associations   Chewing lice

in the lice occurred after speciation in their hosts). Thus, the genetic distance evidence falsifies the cospeciation hypothesis for these *Thomomys–Thomomydoecus* associations, and it appears that gene flow between louse populations persisted long after speciation in their hosts. It is also possible that these associations result from a kind of host switching in which a parasite colonizes a new host that happens to be the sister taxon of the original host of the parasite. If the colonizer then speciates on the new host, the resulting arrangement—sister taxa of parasites found on sister taxa of hosts—could be mistaken for cospeciation (we term this pseudo-cospeciation). Investigators comparing only the branching sequences of host and parasite phylogenies cannot distinguish between pseudo-cospeciation and cospeciation in the absence of genetic distance data.

The use of biochemical genetic evidence to investigate the evolutionary histories of hosts and their parasites allows us to view coevolution from two perspectives: sequence and timing of phylogenesis. Data are gathered independently for hosts and parasites, thus avoiding the nagging problem of circularity in studies of host-parasite coevolution. Patterns of allele-sharing reveal the sequence of phylogenetic events in each group, and comparisons of genetic distances between associated host and parasite lineages provides a single metric to assess the temporal relationship of speciation events in both. Using biochemical methods, we have conducted rigorous tests of the cospeciation

hypothesis in this host–parasite assemblage, and we have failed to falsify it in several cases. We regard this as the strongest evidence yet for cospeciation in a host-parasite assemblage.

We thank D. J. Hafner, R. D. Price and R. M. Sullivan fc assistance. D. W. Foltz, D. P. Pashley and J. V. Remsen J provided helpful criticisms of an earlier draft of this manuscrip This work was supported by a grant from the NSF.

1. Mitter, C. & Brooks, D. R. in *Coevolution* (eds Futuyma, D. J. & Slatkin, M.) 65–98 (Sinaue Sunderland, Massachusetts, 1983).
2. Stone, A. R. & Hawksworth, D. L. *Coevolution and Systematics* (Clarendon, Oxford, 1986
3. Eichler, W. *Annls mag. nat. Hist.* **12**, 588–598 (1948).
4. Lyal, C. H. C. *J. nat. Hist.* **21**, 1–28 (1987).
5. Marshall, A. G. *The Ecology of Ectoparasitic Insects* (Academic, London, 1981).
6. Smolen, M. J., Genoways, H. H. & Baker, R. J. *J. Mammal* **61**, 224–236 (1980).
7. Timm, R. M. in *Coevolution* (ed. Nitecki, M. H.) 225–265 (University of Chicago Press, 1983
8. Harris, H. & Hopkinson, D. A. *Handbook of Enzyme Electrophoresis in Human Geneti* (North-Holland, Amsterdam, 1976).
9. Hafner, M. S., Hafner, J. C., Patton, J. L. & Smith, M. F. *Syst. Zool.* **36**, 18–34 (1987).
10. Honeycutt, R. L. & Williams, S. L. *J. Mammal* **63**, 208–217 (1982).
11. Sneath, P. H. A. & Sokal, R. R. *Numerical Taxonomy* (Freeman, San Francisco, 1973).
12. Nelson, G. & Platnick, N. *Systematics and Biogeography* (Columbia University Press, 1981)
13. Hall, E. R. *The Mammals of North America* (Wiley, New York, 1981).
14. Wilson, A. C., Carlson, S. S. & White, T. J. *A. Rev. Biochem.* **46**, 573–639 (1977).
15. King, J. L. & Jukes, T. H. *Science* **164**, 788–798 (1969).
16. Fitch, W. M. in *Molecular Evolution* (ed. Ayala, F. J.) 160–178 (Sinauer, Sunderland Massachusetts, 1976).
17. Sarich, V. M. *Nature* **265**, 24–28 (1977).
18. Kojima, K., Gillespie, J. H. & Tobari, Y. N. *Biochem. Genet.* **4**, 627–637 (1970).
19. Rogers, J. S. *Univ. Texas Publ.* **7213**, 145–153 (1972).