Disparate Rates of Molecular Evolution in Cospeciating Hosts and Parasites

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DNA sequences for the gene encoding mitochondrial cytochrome oxidase I in a group of rodents (pocket gophers) and their ectoparasites (chewing lice) provide evidence for cospeciation and reveal different rates of molecular evolution in the hosts and their parasites. The overall rate of nucleotide substitution (both silent and replacement changes) is approximately three times higher in lice, and the rate of synonymous substitution (based on analysis of fourfold degenerate sites) is approximately an order of magnitude greater in lice. The difference in synonymous substitution rate between lice and gophers correlates with a difference of similar magnitude in generation times.

Chewing lice of the genera Geomyduscus and Thomomysdocus are obligate ectoparasites of pocket gophers (Fig. 1). Because the entire life cycle of these lice occurs exclusively in the fur of the host, and because different host species rarely interact, each species of louse is normally restricted to a single host species (1). As a result, there is close correspondence between gopher taxonomic boundaries and louse taxonomic boundaries (2). When viewed over large geographic and temporal scales, this restricted distributional pattern of chewing lice on pocket gophers has resulted in phylogenetic histories of lice and gophers that are remarkably similar (3-5).

Although well-documented cases of host-parasite coassociation are rare (3, 6), they are of interest because they permit comparative study of organisms with a long history of parallel evolution. The temporal component of parallel phylogenesis (in which lineages of hosts and their parasites speciate repeatedly at approximately the same time) permits examination of relative rates of evolution in the two groups by comparison of the amount of change each has undergone during their parallel histories. Because the life histories of hosts and their parasites are often profoundly different, studies of molecular evolution in host-parasite assemblages can help answer a broad spectrum of questions relating to the possible effects of generation time, metabolic rate, and other life history parameters on rates of mutation and evolutionary change.

We examined DNA sequence variation in 14 species of pocket gophers and their chewing lice (7) to test for cospeciation and to investigate rates of molecular evolution in this host-parasite assemblage. We sequenced and compared homologous regions of the gene encoding the mitochondrial cytochrome c oxidase subunit I (COI) in both groups (8). Of the 379 nucleotides sequenced for each taxon, 134 positions were variable in pocket gophers and 178 positions were variable in chewing lice (Table 1).

The cospeciation hypothesis predicts that the branching structure of the host and parasite phylogenies will be similar to a degree beyond that expected by chance. This prediction can be evaluated statistically (3, 4). For any particular host-parasite assemblage, confidence in the test of cospeciation can be no stronger than confidence in the phylogenies under comparison. Thus, it is essential that the host and parasite phylogenies accurately estimate the evolutionary history of each group. There are many methods for estimating phylogenies from sequence data (9), each of which uses a different model of nucleotide evolution and potentially yields a phylogenetic hypothesis (a tree) that differs from that estimated with other methods (10). To consider the effects of different evolutionary models on our estimates of phylogeny, we applied multiple methods of analysis (11, 12) to our sequence data. In cases where different methods yielded different results, we retained all host and parasite trees for topological comparison in order to determine whether the inference of cospeciation is warranted and, if so, whether the inference is sensitive to the method of analysis.

All analyses of the pocket gopher sequence data (using different models of DNA sequence evolution) yielded trees that were very similar in overall branching structure. For example, phylogenetic analysis (11) of the COI sequence data for pocket gophers yielded two most-parsimonious trees of equal length (1423 steps). One of these trees (Fig. 2A) was topologically identical to the tree generated by a maximum-likelihood analysis of the same data (12). The other most-parsimonious tree showed only minor differences (13) from the tree shown in Fig. 2A. The general structure of the gopher parsimony tree (Fig. 2A) also was supported by Fitch-Margoliash (Fig. 2B) and neighbor-joining (14) analyses of genetic distances (12). Differences among the trees generated by the parsimony, maximum-likelihood, Fitch-Margoliash, and neighbor-joining analyses of the pocket gopher data were judged nonsignificant by a likelihood ratio test (15). Accordingly, all four trees were retained for topological comparison with the parasite trees. The basic structure of these trees and, in particular, relations within the genera Orthogeomys and Geomys, also are supported by inde-

Table 1. Observed percent of difference (mean ± 1 SD) in various elements of the COI nucleic acid sequence from pocket gophers and their ectoparasitic chewing lice.

<table>
<thead>
<tr>
<th></th>
<th>Gophers</th>
<th>Lice</th>
</tr>
</thead>
<tbody>
<tr>
<td>First position</td>
<td>1.43 (0.49)</td>
<td>2.09 (0.62)</td>
</tr>
<tr>
<td>First position</td>
<td>0.00 (0.25)</td>
<td>0.46 (0.34)</td>
</tr>
<tr>
<td>First position</td>
<td>0.24 (0.18)</td>
<td>0.38 (0.37)</td>
</tr>
<tr>
<td>First position</td>
<td>0.00 (0.00)</td>
<td>0.16 (0.17)</td>
</tr>
<tr>
<td>First position</td>
<td>8.74 (1.65)</td>
<td>9.59 (1.51)</td>
</tr>
<tr>
<td>First position</td>
<td>5.01 (1.67)</td>
<td>7.68 (1.99)</td>
</tr>
<tr>
<td>Total difference</td>
<td>15.64 (3.20)</td>
<td>20.75 (2.31)</td>
</tr>
<tr>
<td>Silent nucleotide</td>
<td>14.75 (3.09)</td>
<td>17.66 (2.32)</td>
</tr>
<tr>
<td>Replacement</td>
<td>0.89 (0.71)</td>
<td>2.67 (1.05)</td>
</tr>
<tr>
<td>Amino acid</td>
<td>2.40 (1.83)</td>
<td>6.85 (2.62)</td>
</tr>
</tbody>
</table>

*Mean and standard deviation based on all pairwise comparisons.
and parasites (3), corroborates independent evidence for speciation in several genera of pocket gophers (Orthogeomys, Geomys, and Thomomys) and their lice (3–5).

Given evidence for speciation, it is possible to test the null hypothesis that pocket gophers and chewing lice have undergone equivalent amounts of genetic differentiation during their parallel histories. It is appropriate that this test be restricted to hosts and parasites that have coevolved, because time since divergence can be assumed to be equal only for host-parasite pairs that show cospeciation (9 host-parasite pairs in Fig. 2A and 10 host-parasite pairs in Fig. 2B) (24). We first compared maximum-likelihood distance matrices for cospeciating gophers and lice using Mantel’s test (25), which showed a highly significant (P < 0.01) association between genetic distances in corresponding hosts and parasites. This test demonstrates that evolutionary rates in gophers and lice are significantly correlated, regardless of tree structure. To test for equality of rates between gophers and lice, we compared maximum-likelihood branch lengths for all possible combinations of cospeciating taxa (24, 26). In all cases, Wilcoxon sign-rank tests showed that louse branches were significantly longer than gopher branches (P < 0.003 in each case). Given this significant difference, we used Model II regression analysis of the louse COI data yielded three most-parsimonious trees of equal length (4208 steps). One of these trees (Fig. 2A) was topologically identical to the tree generated by a maximum-likelihood analysis of the same data. The two remaining parsimony trees showed only minor differences (involving one species in each case) from the tree in Fig. 2A (19). The Fitch-Margoliash analysis (Fig. 2B) and the neighbor-joining analysis (20) yielded louse trees very similar to those generated by the parsimony analysis. Differences among the trees generated by the parsimony, maximum-likelihood, Fitch-Margoliash, and neighbor-joining analyses of the louse data were judged nonsignificant by a likelihood ratio test (15). Accordingly, all five louse trees were retained for topological comparison with the host trees. The basic structure of the louse trees and, in particular, relations among lice hosted by species of Orthogeomys and Geomys, also are supported by independent phylogenetic studies of allozymes (3, 5, 21).

The COMPONENT program (22) determined if the fit between observed parasite and host trees was significantly better than the fit between the parasite tree and trees drawn at random from the set of all possible host trees (4). For each of 20 pairwise comparisons (four host trees and five parasite trees), the observed degree of fit between the gopher and louse trees was significantly better (P < 0.01) than the fit between the louse tree and 10,000 randomized gopher trees (23). These results, which are robust to the method of phylogenetic inference and to the evolutionary models used, falsify the null hypothesis of chance similarity between the host and parasite trees. Although this evidence is consistent with the hypothesis of cospeciation, the concordant phylogenies might instead result from dispersal, extinction, or incomplete sampling of closely related taxa (4). However, only the cospeciation hypothesis predicts temporal congruence of host and parasite speciation events, which (given roughly time-dependent molecular change in each group) would result in a significant relation between measures of molecular differentiation in the host and parasite trees. We demonstrate below that our molecular data are consistent with this prediction. This finding, which requires no assumptions about rate similarity between hosts

![Fig. 2](https://example.com/f2.png)
then change at these sites should fit a molecular clock model (30). Accordingly, we tested all possible combinations of cospeciating gophers (Orthogeomys only) and their lice (24) for significant departure from clocklike behavior, using the log-likelihood ratio test (12). In all cases, the data were consistent with molecular-clock assumptions, which indicates that substitutions within gophers and within lice accumulate in a roughly time-dependent fashion. Wilcoxon sign-rank tests showed that louse branches were significantly longer than gopher branches in four of the six possible comparisons (P < 0.05 in each case). We used Model II regression analysis (through the origin) to quantify the relation between gopher and louse branch lengths. Slopes of the regressions (Fig. 3B) ranged from 0.66 to 1.13 (with a mean of 1.04), which indicates that the estimated rate of silent substitution for this gene region is approximately an order of magnitude greater in chewing lice than in pocket gophers. Evidence for a higher rate of substitution in lice appears to be independent of the evolutionary model employed, although the magnitude of the rate difference is sensitive to certain parameters of the model (31).

Viewed together, the analysis of all nucleotide substitutions (Fig. 3A) and the analysis of substitutions at fourfold degenerate sites (Fig. 3B) provide insight into the dynamics of molecular evolution for this gene region in the species studied. The analysis of all substitutions indicates that the overall rate of evolutionary change is approximately three times greater in chewing lice than in their hosts (Fig. 3A). Likewise, the means of all pairwise replacement differences for nucleotides and amino acids are approximately three times greater in lice than in gophers (Table 1). In contrast, the analysis of nucleotide substitutions at fourfold degenerate sites indicates that rates of silent substitution in this gene region are roughly 11 times greater in lice than in gophers (Fig. 3B). The fact that this 11-fold rate difference is not evident when all substitutions are considered is probably the result of selective constraints on replacement substitutions. High levels of functional constraint on the COI enzyme have been reported in other organisms (28).

The 11-fold difference in rates of synonymous substitution in Orthogeomys gophers and their lice (Fig. 3B) cannot be explained by transition bias or nucleotide frequency bias. Because silent substitutions at the fourfold degenerate sites show clocklike behavior, it is likely that they are neutral or nearly neutral (30). Several possible mechanisms could account for this rate difference, including mutation rate differences caused by possible differences in gene order that affect vulnerability to mutation (32), differences in metabolic rate or general metabolic physiology, generation-time differences, or other factors correlated with body size (33). Alternatively, this rate difference could be caused by mechanisms that are independent of mutation rate, such as codon bias and other constraints on the translational apparatus, or differences in DNA repair efficiency. It is perhaps important that this 11-fold rate difference is accompanied by a similar difference in generation time between gophers and lice (approximately 1 year in gophers and 40 days in lice) (34). If the observed rate difference results from an underlying difference in mutation rate, then generation time may explain this difference. However, mutation rates are more likely to be influenced directly by nucleotide generation time than by organism generation time (33). As such, our study suggests that each organismal generation is equivalent to equal numbers of nucleotide generations in pocket gophers and chewing lice. If the 11-fold rate difference reflects a similar difference in mutation rate, then these findings are consistent with the neutral theory of molecular evolution (30), because once the data are corrected for the difference in generation time, they suggest equal rates of mutation per generation in distantly related groups of animals.

REFERENCES AND NOTES


11. We used PHYLIP (Phylogeny Inference Package, Department of Genetics, University of Washington, Seattle) and the neighbor-joining method (J. E. Felsenstein, J. Mol. Evol. 17, 351 (1980)) analyses of the sequence data. Empirical base frequencies and maximum observed transition biases (10:1 for gophers and 17:1 for lice) were used in the maximum-likelihood analyses. The Fitch-Margoliash and neighbor-joining analyses were based on distance matrices corrected with the Kimura two-parameter model (substituting maximum observed transition bias values for the default (2.1) value) (M. Kimura, J. Mol. Evol. 16, 111 (1980)). We repeated each analysis at least 10 times, varying the input order of taxa using the jumble option of PHYLIP.

12. In the alternative parsimony tree for pocket gophers, the phylogenies of the Gopherus specioius and Z. trichopus groups were reversed and the two species of Cymomys were not depicted as sister taxa.

13. The neighbor-joining tree differed from the Fitch-Margoliash tree (Fig. 2B) only in the arrangement of P. rutilus (immediate basal to the five-taxa clade containing C. rutulus and Z. trichopus) at the base of the tree containing C. castanops, C. merriami, and P. rutilus.


18. In the second parsimony tree, the loess G. acutus was linked with the eleutheromys-nevadensis clade, rather than the tenuis-argentipes clade (as in Fig. 2A). The third tree showed G. thomomys near the root of the tree between the outgroups T. minor and T. barbara.

19. The neighbor-joining tree was identical to the Fitch-Margoliash tree (Fig. 2B), except that G. thomomys and G. personatus were linked as sister taxa (as in the parsimony and maximum-likelihood trees, Fig. 2A) and positioned basal to the G. chapini branch.


22. The test was based on the criterion of minimum number of independent losses necessary to reconcile the host and parasite trees (22). Sixteen of the 20 pairwise comparisons were found to be statistically significant according to the criterion of "number of leaves added" (22). Number of leaves added is calculated as one-half the number of "items of error" (22). No preference was suggested for a major axis model, which does not permit constraints on the regression line, and for the multivariate regression model. The number of independent losses is calculated from the major axis model.

23. For this analysis, only one taxon was treated as an outgroup. In this tree, Thrommys and Harmonomys are sister taxa.

24. The trees (Fig. 2A) were reduced to the largest number of taxa that showed identical branching patterns in the hosts and parasites. All possible combinations of cospeciating taxa were compared. For example, there are four possible combinations of nine cospeciating taxa in Fig. 2A (that is, the nine-taxon gopher tree can include either O. underwoodii or O. cavator (but not both). There are 2 possible combinations, 10 of 10 cospeciating taxa in Fig. 2B (that is, the tree can include either G. brevicaudus or G. parsoniense, but not both). Because most of the phylogenetic analyses involved branches near the base of the trees, only terminal and subterminal branches were compared for gophers and lice.}

25. N. Mantel, Cancer Res. 27, 200 (1967). We calculated distance matrices using the maximum-likelihood model (22). For the 9 cospeciating taxa in Fig. 2A, a phylogenetic tree was constructed for each of the distance matrices (Fig. 2B). In Fig. 2B, t = 4.289, P < 0.001. Degrees of freedom were adjusted to n-1, where n = number of taxa compared (5).

26. Trees for cospeciating taxa (24) were input as user trees into PHYLIP (12) in order to estimate maximum-likelihood branch lengths. Empirical estimates of transition bias, nucleotide frequencies, and positional bias (first-second-third codon position: 5:1:40 for gophers and 5:1:21 for lice) were incorporated into the maximum-likelihood trees (22). We recognize that the parameters used in maximum-likelihood models will influence rate estimates (K. Fukami-Kobayashi and Y. Tatsukawa, Int. J. Evol. Biol. 4, 406 (1987)) and that the parameters of the model may differ from the model used in previous morphological and allozyme analyses of the same taxa (10:1 for gophers and 17:1 for lice).