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A Different Tempo of Mitochondrial DNA Evolution in Birds and Their Parasitic Lice

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A phylogeny for the lice (Insecta: Phthiraptera: genus *Dennyus*) parasitic on swiftlets (Aves: Collocalliinae) was constructed based on mitochondrial cytochrome *b* DNA sequences. This phylogeny is congruent with previous phenetic analyses of morphometric data for the lice. Comparison with a previously obtained phylogeny for the hosts indicates some degree of cospeciation. These cospeciation events are used to compare relative rates of evolution in the birds and their lice for the same segment of the cytochrome *b* gene. Cytochrome *b* is evolving two to three times more rapidly in lice than in birds, and louse cytochrome *b* is highly divergent compared to that of most other insects. Although generation time has been suggested as an explanation for the disparity in evolutionary rates between lice and their hosts, we suggest that the small effective population sizes of lice coupled with founder events occurring during transmission to new host individuals may be an important factor. © 1998 Academic Press

INTRODUCTION

A central question in the evolutionary study of any host–parasite assemblage is the relative antiquity of the host–parasite association; is the parasite a recently acquired "souvenir" or an "heirloom" inherited from the host's ancestor (Sprent, 1970)? In the latter case, if a parasite is specific to a single host lineage then, as that host lineage speciates, populations of the parasite on the descendant host species may themselves speciate. If parasites only speciate when the hosts speciate, and never go extinct independently of their hosts, this parallel cladogenesis or "cospeciation" (Brooks, 1988) will yield host and parasite phylogenies that are mirror images of each other. However, if other processes such host switching and extinction occur then the relationship between the evolutionary trees for the two members of the association will be more complex and hence more difficult to unravel (Lyal, 1986; Page, 1994b).

Parasites may speciate independently of their hosts, go extinct, or switch to different hosts. These processes and the terminology used in this paper are illustrated in Fig. 1a. Independent speciation by parasites can result in more than one lineage of closely related parasites on the same host. This is termed a "duplication" by analogy with gene duplications (Page, 1993b). If the two descendant parasite lineages subsequently cospeciate with the host then the host clade will harbor two parallel sets of parasites (Fig. 1b). Following Fitch's (1970) terminology for genes, we describe the two sets of parasites as "paralogous"; members of the same lineage of parasites are "orthologous" (Page, 1993b).

Recent advances in molecular systematics facilitate rigorous analysis of the history of host–parasite assemblages, in part by providing mutually independent phylogenies for host and parasite. Because cospeciation is inferred when the topologies of host and parasite phylogenetic trees are more similar than would be expected by chance (Hafner and Nadler, 1990; Page, 1993c) it is vital that host and parasite evolutionary histories are inferred independently. The use of molecular data can reduce the extent to which knowledge of the classification of one member of the association influences that of the other, as well as minimizing the possibility that the phylogenetic analysis is misled by host effects on parasite morphology (Downes, 1990).

Molecular data are also attractive because they provide a common yardstick for comparing evolutionary character change (anagenesis) in taxonomically (and morphologically) disparate groups (Page, 1993c). Homologous genes can be readily found in most taxa, whereas homologous morphological features may be extremely difficult to find in, say, a vertebrate and its insect parasite. Furthermore, if the molecular divergence in host and parasite is approximately clock-like then specific hypotheses about the relative antiquity of

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a particular host–parasite association can be tested (Page *et al.*, 1996).

At the same time, cospeciating host–parasite assemblages provide a unique opportunity to compare the evolutionary rates of dissimilar organisms that have lived in intimate association over long periods of time (Hafner and Page, 1995; Page and Hafner, 1996). Calibrating the absolute rate of evolution in a single clade of organisms can be problematic if the fossil record of that clade is poor. However, if that clade has cospeciated with a clade that does have a fossil record, then those fossils can also be used to calibrate the rate of evolution in the clade that lacks fossils of its own. This is because if a pair of host and parasite lineages have cospeciated then those lineages are of the same age (Fig. 2a). This approach of calibrating one lineage using another, associated, lineage has been applied to endosymbiotic bacteria of aphids (Moran *et al.*, 1993) and cockroaches (Bandi *et al.*, 1995) and alpha herpes viruses of vertebrates (McGeoch *et al.*, 1995). Even if neither member of a cospeciating association has an adequate fossil record we can still obtain a measure of the relative rate of evolution in the two lineages because cospeciation implies that there are events of the same age in the two clades, even if we do not know

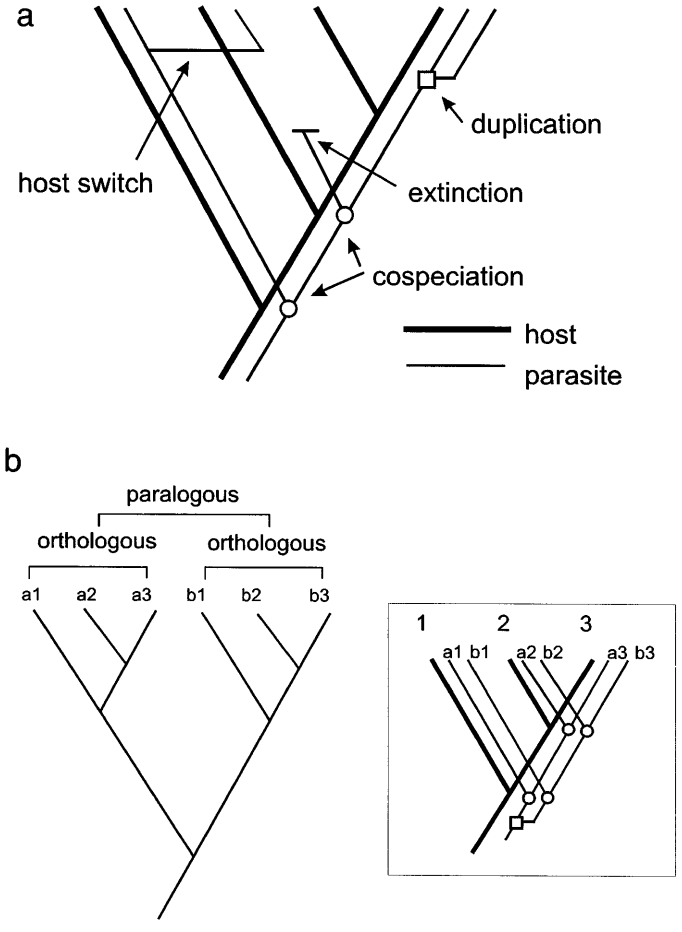

FIG. 1. (a) Host and parasite phylogenies and the terminology used in this paper. Cospeciation is parallel speciation by host and parasite. Parasites may also go extinct or transfer from one host to another (a host switch). A parasite speciating independently of its host with both descendants remaining on the host is termed a duplication. (b) The terms "orthologous" and "paralogous" can be applied to parasites (Page, 1993b) as well as to genes (Fitch, 1970). If a parasite lineage speciates independently of its host, and both its descendant lineages (a and b) remain on the host and then subsequently cospeciate, then hosts 1–3 will each have two parasites, one from lineage a and one from lineage b. Parasite clades a and b are paralogous; parasites a1–a3 are orthologous, as are parasites b1–b3.

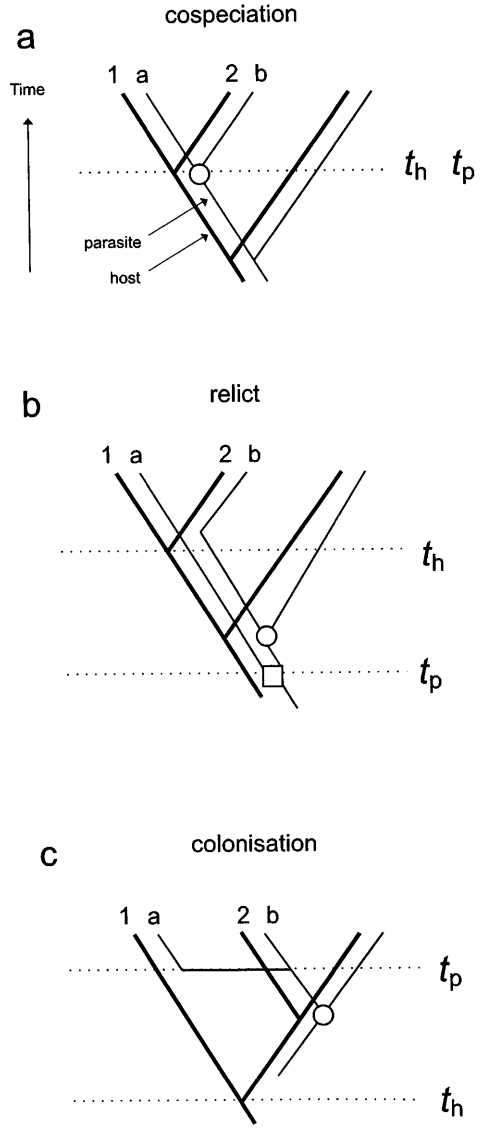

FIG. 2. Three possible histories for three hosts and their parasites. In each case the times of divergence between a pair of hosts (1 and 2) and the equivalent pair of parasites (a and b) is indicated by t_h, and t_p, respectively. Only if host and parasite have cospeciated (a) will $t_h \cong t_p$. If the parasites are relicts (b) then the hosts will be younger than their parasites. If one or other of the hosts has been recently colonized (c) then the hosts will be older than their parasites. See Fig. 1a for key to symbols used.

the absolute age of those cospeciation events. Relative rates of molecular evolution have been measured in this way between chewing lice and mammals (Hafner *et al.*, 1994) and endosymbiotic bacteria and aphids (Moran *et al.*, 1995).

Calibrating rates of evolution in hosts and parasites depends crucially on the presence of cospeciation and on correctly identifying which events in the two clades represent cospeciation. It is not enough simply to compare divergence in pairs of hosts and their parasites. If the parasites are older than their hosts, then greater divergence between a pair of parasites may reflect greater antiquity relative to their hosts, rather than a difference in rate of evolution (Fig. 2b). This is analogous to the mistake of using divergence between paralogous genes as a measure of time of divergence between organisms; valid comparisons of host and parasite divergence must use "orthologous" parasites (Page, 1993b). Conversely, if the parasites have not cospeciated with their hosts but instead have switched hosts then the parasite pair may be much younger than their hosts (Fig. 2c). In this study we use molecular phylogenies to determine whether there is evidence for cospeciation between a group of birds and their lice and to investigate the relative rates of evolution in these two taxonomically distant organisms.

LICE

Lice are wingless insects that are permanent, obligate ectoparasites of birds and mammals, i.e., they complete their entire life cycle on the body of the host (Barker, 1994; Marshall, 1981). On the basis of this close ecological association and the apparent correspondence between some avian and louse taxonomic groupings it appears that there has been extensive parallel cladogenesis between lice and their hosts (Clay, 1949; Mauersberger and Mey, 1993). Despite rapid advances in avian and mammalian systematics (Allard *et al.*, 1996; Sheldon and Bledsoe, 1993; Sibley and Ahlquist, 1990) there have been few phylogenetic hypotheses of cospeciation between particular groups of birds or mammals and their lice (Hafner and Nadler, 1988; Kim, 1988; Paterson and Gray, 1997; Paterson *et al.*, 1993, 1995). One reason for this limited evidence bearing on whether lice have cospeciated with their hosts is the need for robust phylogenies for both groups. In addition to this obvious requirement, information on the relative ages of host and parasite lineages can greatly assist determining whether the two have cospeciated (Page *et al.*, 1996). Given the poor fossil record for most parasites, information on age is most likely to come from molecular data.

To date there is only one published study where homologous DNA sequences from both hosts and lice have been obtained and compared. Hafner *et al.* (1994) reported an order of magnitude difference between lice and gophers in the rate of substitution at the third codon position in mitochondrial cytochrome oxidase I and suggested that the more rapid evolution of the lice may reflect their shorter generation time relative to that of their hosts. Subsequent independent reanalyses of these data (Huelsenbeck *et al.*, 1997; Page, 1996a) support Hafner *et al.*'s conclusion of a more rapid rate of substitution in lice, but suggest that the disparity in rates is approximately 2.5 rather than an order of magnitude. As this is the only published study of louse DNA to date it is not known whether this disparity applies to other mammalian or bird lice and their hosts.

As part of a study of host–parasite interactions between swiftlets (Aves: Collocalliinae) and their lice (Insecta: Phthiraptera) we obtained mitochondrial cytochrome *b* DNA sequences from the lice, with the goal of constructing a phylogeny for these taxa and comparing it to a phylogeny for the birds constructed previously by Lee *et al.* (1996). Molecular data were employed because swiftlet lice, which belong to the subgenus *Collodennyus* of the genus *Dennyus*, are morphologically very similar and have few discrete morphological characters suitable for cladistic analysis. This lack of discrete characters prompted Clayton *et al.* (1996) to resort to a phenetic analysis to generate an initial hypothesis of relationships (Fig. 3). This analysis separated the swiftlet louse subgenus *Collodennyus* from representatives of the subgenus *Dennyus* found on swifts and supported the division of *Collodennyus* into two species-groups: *distinctus* and *thompsoni*. The *thompsoni* species-group was subdivided into two distinct clusters, the *thompsoni* and *francicus* subgroups. Clayton *et al.* (1996) separated the *distinctus* species-group into three subgroups (*distinctus, ellioti,* and *emersoni*); however, the phenetic analyses did not consistently resolve relationships among these three subgroups.

The purpose of this study was to address four questions: (i) what is the phylogeny of *Dennyus* lice from swiftlets? (ii) is there evidence that *Dennyus* lice have cospeciated with swiftlets? (iii) what are the relative rates of mtDNA evolution in swiftlets and lice? and (iv) how does the rate of louse molecular evolution compare to that in other insects?

METHODS

Specimens

Chewing lice were collected when their swiftlet hosts were sampled for the study by Lee *et al.* (1996). Most samples were collected in the field by D.H.C. The collection of *Dennyus* (*Collodennyus*) lice from swiftlets (genera *Aerodramus* and *Collocalia*) was divided into two sets; one was used by Clayton *et al.* (1996) for morphological taxonomic description, while the other was used in this study. Thus the lice used in both morphological and molecular phylogenetic analyses, as well as the samples used for the molecular phylogenetic

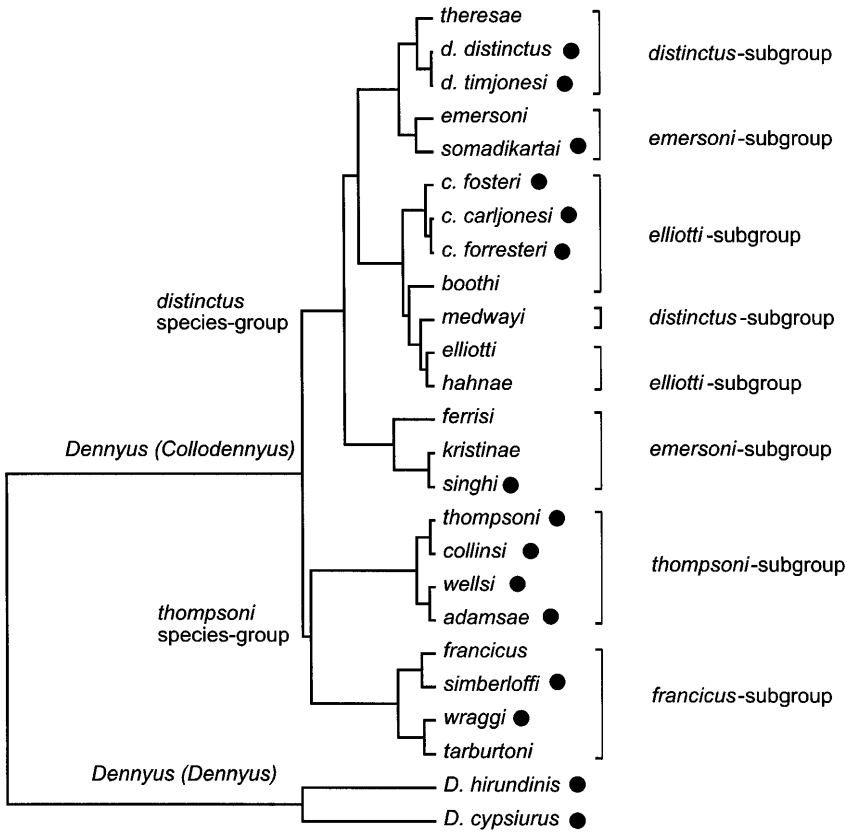

FIG. 3. Relationships among *Dennyus* lice from swifts and swiftlets based on cluster analysis of morphometric data (Clayton *et al.*, 1996). Lice from which cytochrome *b* mtDNA sequences where obtained in this study are indicated by ●.

study of the hosts (Lee *et al.*, 1996), were from the same populations. Additional lice from the subgenus *Dennyus* (*Dennyus*) were collected from the swifts *Apus apus*, *Cypsiuris balasiensis*, and *Chaetura vauxi* to serve as outgroups.

Live birds were searched for chewing lice using the visual examination method described in Clayton and Walther (1997). Any lice encountered were removed with forceps and placed in an Eppendorf tube, where they were starved for 12–24 h to allow digestion of ingested host material. They were then frozen in a portable liquid nitrogen tank (Cryopak, Taylor-Wharton) and stored at −70°C in the laboratory. When liquid nitrogen was unavailable, the lice were killed in absolute ethanol and stored in the laboratory at 4°C following recommendations by Post *et al.* (1993).

Swiftlet lice are morphologically very similar, making positive identification impossible without examining slide-mounted specimens of adult lice under a compound microscope. Unfortunately, specimens for molecular work cannot be slide mounted without degrading their DNA. In addition, owing to limited numbers of specimens of certain taxa, some of the lice we sequenced were nymphs that cannot be identified reliably from morphology alone. Thus, lice for molecular study were identified using one of three criteria. First, if only one *Dennyus* had been reported from a particular host, then lice sequenced from that host were assumed to be that taxon. Second, if more than one louse species was known to occur on a host, and the lice belonged to different species-groups (Fig. 3), then unmounted adult specimens were assigned to species-group based on the gross shape of the lateral preantennal margins of the head, which is easily determined under a dissecting microscope (Clayton *et al.*, 1996). Finally, three species of swiftlets in this study are host to multiple taxa of lice from the same species-group: *Collocalia esculenta cyanoptila* is host to three *distinctus* species-group lice, *C. linchi* is host to two of these same taxa, and *Aerodramus maximus* is host to two members of the *thompsoni* species-group. Sequences from lice collected from *C. e. cyanoptila* at Ampang Reservoir (GenBank Accession numbers U96415 and U96416) were assigned to *D. distinctus timjonesi*, as this is the type locality of that subspecies and no other louse has ever been recovered from that location (Clayton *et al.*, 1996). By the same argument, sequence U96419 from Bogor, Java, was assigned to *D. somadikartai*. The two remaining sequences from lice on *C. e cyanoptila* from Sandakan (U96417 and U96418) were assigned to *D. d. distinctus* and *D. somadikartai*, respectively, based on their position relative to the sequences from Ampang and Bogor in preliminary trees we constructed from the sequence data. Se-

TABLE 1

Louse Taxa Sequenced, Together with Host, Collection Locality, and GenBank Accession Number

Louse taxon	Life history stage	Host[a]	Locality	GenBank Accession No.
hirundinis	Adult	*Apus apus*	Oxford, UK	U96434
cypsiurus	Nymph	*Cypsiurus balasiensis*	UM Campus, Kuala Lumpur, Malaysia	U96433
vauxi	Adult	*Chaetura vauxi*	Union County, Oregon	U96432
distinctus distinctus	Nymph[b]	*Collocalia esculenta cyanoptila*	Sandakan, Sabah, Malaysia	U96417
distinctus timjonesi	Nymph[c]	*Collocalia esculenta cyanoptila*	Ampang Reservoir, near Kuala Lumpur, Malaysia	U96415
	Nymph[c]	*Collocalia esculenta cyanoptila*	Ampang Reservoir, near Kuala Lumpur, Malaysia	U96416
somadikartai	Adult[c]	*Collocalia linchi*	Bogor, Java, Indonesia	U96419
	Nymph[b]	*Collocalia esculenta cyanoptila*	Sandakan, Sabah, Malaysia	U96418
carljonesi carljonesi	Adult[c]	*Aerodramus fuciphagus vestitus*	Gomantong Caves, Sabah, Malaysia	U96420
carljonesi forresteri	Adult[c]	*Aerodramus francicus*	Mauritius, Indian Ocean	U96422
carljonesi fosteri	Adult[c]	*Aerodramus elaphrus*	Seychelles, Indian Ocean	U96421
singhi	Adult[c]	*Aerodramus spodiopygius assimilis*	Nasinu Cave, Suva, Fiji	U96423
thompsoni	Adult[b]	*Aerodramus maximus*	Balambangan Is, Sabah, Malaysia	U96425
	Nymph[b]	*Aerodramus maximus*	Gomantong Caves, Sabah, Malaysia	U96424
collinsi	Nymph[c]	*Aerodramus brevirostris vulcanorum*	Tangkuban Prahu, Java, Indonesia	U96426
adamsae	Nymph[b,c]	*Aerodramus t. terraereginae*	Tully Gorge, Queensland, Australia	U96427
	Nymph[b]	*A. t. chillagoensis*	Chillagoe, Queensland, Australia	U96428
wellsi	Adult[c]	*Aerodramus salanganus*	Gomantong Caves, Sabah, Malaysia	U96429
simberloffi	Adult[b]	*Aerodramus fuciphagus vestitus*	Gomantong Caves, Sabah, Malaysia	U96431
wraggi	Nymph[b]	*Aerodramus spodiopygius assimilis*	Nasinu Cave, Suva, Fiji	U96430

[a] Host classification follows Chantler and Driessens (1995), except for generic nomenclature, which follows Brooke (1972).
[b] Sequence identified by relationship to other sequences.
[c] Specimen sequenced was obtained from the type locality.

quences from lice collected from *A. maximus* were identified based on the relationships of these sequences to other *thompsoni* species-group lice and by comparison with the phenogram shown in Fig. 3.

Sequencing

Genomic DNA was extracted from single lice using the methods described in Lee *et al.* (1996). A 700-bp fragment of louse cyt *b* mtDNA was amplified by polymerase chain reaction (PCR) using the degenerate primers (L11122) 5′-GAAATTTTGGGTCWTTRCTNGG-3′ and (H11823) 5′-GGCATATGCGAATARGAARTATCA-3′. The numbers in the prefix to the primers indicate their 3′ end positions according to the honeybee cyt *b* mtDNA (GenBank Accession No. L06178), and L and H are the light and heavy strands, respectively. Primer L11122 was designed from insect sequences in GenBank and a 810-bp cyt *b* mtDNA sequence from *Dennyus hirundinis* (GenBank Accession number U96434). This fragment was obtained by PCR amplification using the avian primers of Lee *et al.* (1996) and sequenced after cloning into pCR-Script (SK^+) (Stratagene). By designing a louse-specific primer, amplification of host material was avoided. The primer H11823 is identical to the primer H15709 used by Lee *et al.* (1996) for the PCR amplification of avian cyt *b* mtDNA.

DNA extractions and PCR preparations were carried out under a laminar blowout flow-hood (Microflow). A set of pipettors was reserved for PCR preparation work, and sterile filter protected pipette tips (Anachem) were used to reduce the risk of cross-contamination by aerosol carryover. Double-stranded PCR amplifications with biotinylated primers (synthesized by Genosys) were performed in 25-μl reactions containing 50 ng of each primer, 0.94 units of *Taq* polymerase (Promega), 2.5 μl of 10× *Taq* buffer with 1.5 mM $MgCl_2$ (Promega), 1.6 μl of 25 mM dNTPs, and 1–3 μl extracted genomic DNA. Negative controls were included and all PCR mixtures were overlaid with 25 μl of mineral oil. The reaction began with denaturation (94°C for 1 min) followed by 30 cycles of annealing at 52–54°C (1 min), template extension at 72°C (1 min), and denaturation at 92°C (1 min). A final annealing and extension step of 52–54°C for 5 min and 72°C for 5 min completed the reaction.

Manual direct sequencing of the PCR-amplified fragment produced the 505 bp of cyt *b* mtDNA sequences listed in Table 1 and Fig. 4. Internal sequencing primers designed using *D. hirundinis* and other insect cyt *b* mtDNA sequences were (L11557) 5′-CGATTTTTTACWCTHCAYTA-3′ and (H11607) 5′-TCTTCCYGTDTCRTGTAA-3′. The biotinylated PCR products were bound to streptavidin-coated paramagnetic beads (Dynal), denatured to leave a single-stranded template, and sequenced (Sequenase 7-deaza-dGTP sequencing kit, USB; T7 DNA polymerase, Pharmacia). The sequences have been submitted to GenBank (Accession numbers U96415-U96434, Table 1).

Sequence Analysis and Phylogeny Inference

Sequences were aligned by eye. Analyses of base composition and transition/transversion ratios were

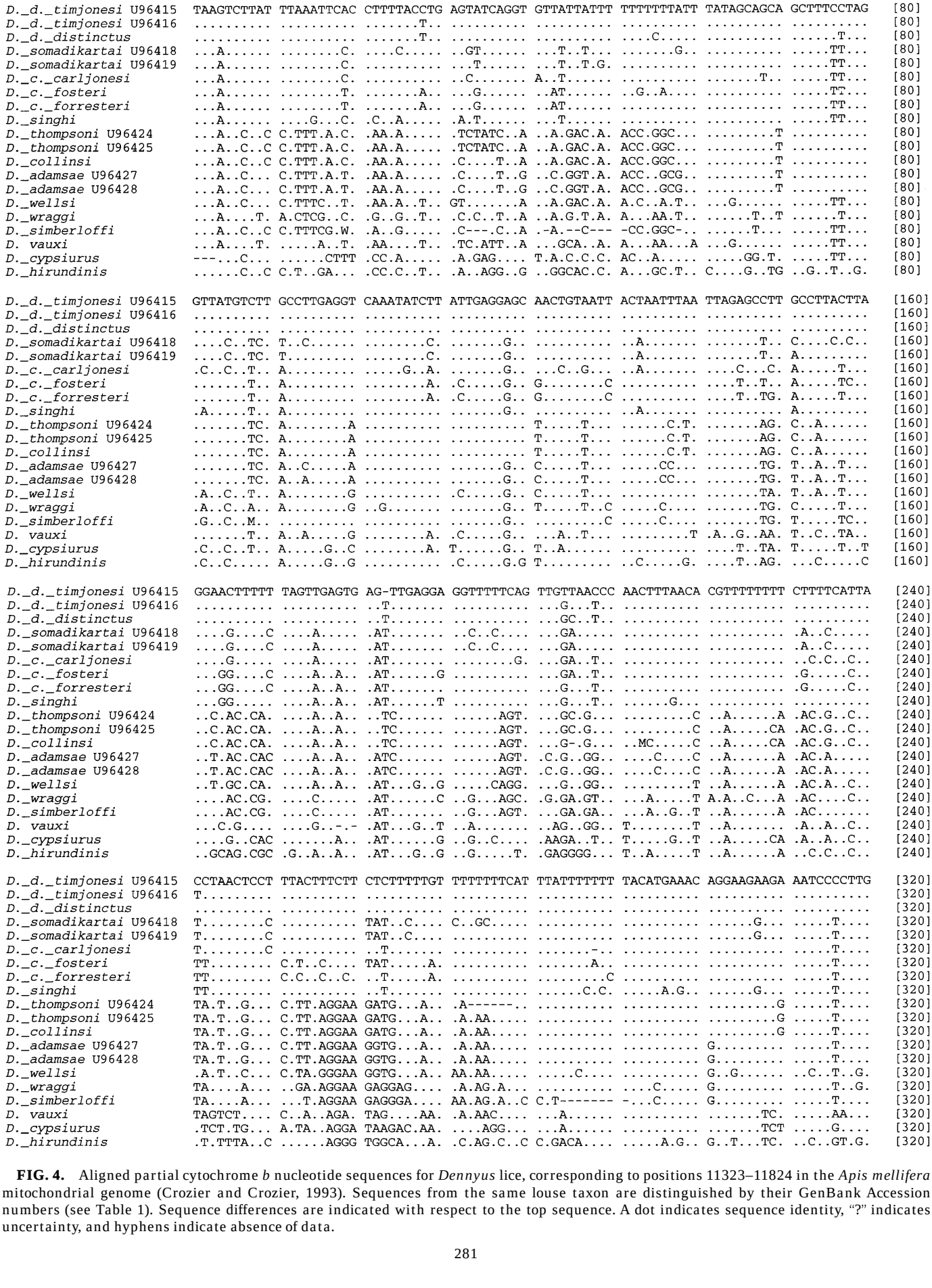

FIG. 4. Aligned partial cytochrome *b* nucleotide sequences for *Dennyus* lice, corresponding to positions 11323–11824 in the *Apis mellifera* mitochondrial genome (Crozier and Crozier, 1993). Sequences from the same louse taxon are distinguished by their GenBank Accession numbers (see Table 1). Sequence differences are indicated with respect to the top sequence. A dot indicates sequence identity, "?" indicates uncertainty, and hyphens indicate absence of data.

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D._d._timjonesi U96415  GACTTGGAAA AAATAGAGAT AAAGTTTTTT TCCATCCTTT TTTTTCTTAT AAAGACGTTC TTGGTTTTTT TTTTTTTTTT  [400]
D._d._timjonesi U96416  .......... .......... .......... .......... .......... .......... .......... ..........  [400]
D._d._distinctus        .......... .......... .......... .......... .......... .......... .......... ..........  [400]
D._somadikartai U96418  .......T.. ...C...... .......... .......... ....A...TA ......T... ....C..... .......G..  [400]
D._somadikartai U96419  .......T.. ...C...... .......... .......... ....A...TA ......T... ....C..... .......G..  [400]
D._c._carljonesi        .......... ...C..G... .......... .T.......A ....A..ATA .....TTA.A ........G. .C........  [400]
D._c._fosteri           .......C.. ......G... ..G....A.. .T.......A C...A...TA ..G..TTA.. ....A...A. .......A..  [400]
D._c._forresteri        .G.....C.. .......... ..G....A.. .T.......A ....A..ATA ..G..TT... ........C. .......G..  [400]
D._singhi               .......T.. .......... .......... .T........ ....A...TA .....TT..T .C......A. ..........  [400]
D._thompsoni U96424     .T..A..... ...C...... ..GA...... .......... ......C.T. .....TT.AA .......... A.........  [400]
D._thompsoni U96425     .T..A..... ...C...... ..GA...... .......... ......C.T. .....TT.AA .......... A.........  [400]
D._collinsi             .T..A..... ...C...... ..GA...... .......... ......C.T. .....TT.AA .......... A.........  [400]
D._adamsae U96427       .TT.A..... ...C..G... ..GA...... .......... ......C.T. .....TT.AA .......... ..........  [400]
D._adamsae U96428       .TT.A..... ...C..G... ..GA...... .......... ......C.T. .....TT.AA .......... ..........  [400]
D._wellsi               .TT.A..... ...C...... ..GA...... .T........ ........TC .....TT.GA ........A. ..........  [400]
D._wraggi               .T.....T.. G..C..G... .......... .T.......A ..A.....T. ......T.AA .C..G..... ..........  [400]
D._simberloffi          .T.....T.. .......... ..G.------ ---------- ---------- ---------- ---------- ----------  [400]
D. vauxi                ..T.A..... .......... ......A.A. .T.......A C...A.AAT. ..G..T...A ........A. .C.......A  [400]
D._cypsiurus            ..T.A..... .......... ...A...... .T........ ....A.A.TA .....T..AT .A..A...A. ...A...AG.  [400]
D._hirundinis           .CT.GA.G.. ...C..T..C ..G..C.... ....C..A.. ......C... .....TT..T .A..G...AG A......GGA  [400]

D._d._timjonesi U96415  TTATTTTTAT TTTTATTTGG ATTAAAATTT CCTACTTTTT TTWWGGATYC RRAWAATTTT TTAATTGCAA ATCCAATA--  [480]
D._d._timjonesi U96416  ..T.....T. ....T..... T......--- ---------- ---------- ---------- ---------- ----------  [480]
D._d._distinctus        ..T.....T. ....T..... T.....---- ---------- ---------- ---------- ---------- ----------  [480]
D._somadikartai U96418  ..T....KG. ....T...-- ---------- ---------- ---------- ---------- ---------- ----------  [480]
D._somadikartai U96419  ..T....GT. ....T...A. ..G....AA. TGCCAAAC.. ..TTA...A. TG.C.C...A ..C--...T. .....T..GT  [480]
D._c._carljonesi        ..T...A.T. C.A.T.C.A. GCA.....CC ---------- ---------- ---------- ---------- ----------  [480]
D._c._fosteri           .AT....... ..A.T...A. .........C .......... ..ATA...T. AG.T....GA A......... ........AC  [480]
D._c._forresteri        .AT....... ..A.T...A. .........C .......... ..ATA...T. AG.T....GA A......... ........AC  [480]
D._singhi               ..C.G...G. ....T..AA. G......... .....C.... ..TA..T.CG GG.A...... AA........ ....C...AC  [480]
D._thompsoni U96424     ..T.------ ---------- ---------- ---------- ---------- ---------- ---------- ----------  [480]
D._thompsoni U96425     ....CCA.C. .A..T...T. T......... ...GTGG.G. .CCT....T. TG.C...... .....G..T. ....T...GT  [480]
D._collinsi             ....CCA.C. .A..T...T. T......... ...GT.G.G. .CCT....T. TG.C...... .....G..T. ....T...GG  [480]
D._adamsae U96427       ......A.T. ....T...T. T..G...... ....TCG.G. ..CT....T. TG.C...... .......... ....T...GT  [480]
D._adamsae U96428       .WW..WW.T. ....T...TT T..R.----- ---------- ---------- ---------- ---------- ----------  [480]
D._wellsi               A.G.....T. ....T..A.. T.....G... ..CTTGGA-- ---------- ---------- ---------- ----------  [480]
D._wraggi               ..CG....T. .CG..A.AAC T....GG... ...TT..... CAAA..T.-- ---------- ---------- ---------- ----------  [480]
D._simberloffi          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------  [480]
D. vauxi                A.T....... ..G..C.CTC T..T...C.. ..A.A..A.. ..CTT...T. TG.T...... .......... ....T...AT  [480]
D._cypsiurus            ..TA...... ....C..CA. .A.......G ..A.AC.... ..TTA...A. TG.C..C... A.TC....T. .....T..GT  [480]
D._hirundinis           C..C.CC..A ....T...A. GA......A. ..C.A.C.C. ..CTT...G. CG.C..C.AC AGCG.G..T. ....C...AC  [480]

D._d._timjonesi U96415  -ACGC-AGTT CATATTCA-C CTGA-   [505]
D._d._timjonesi U96416  ---------- ---------- -----   [505]
D._d._distinctus        ---------- ---------- -----   [505]
D._somadikartai U96418  ---------- ---------- -----   [505]
D._somadikartai U96419  C.TA------ ---------- -----   [505]
D._c._carljonesi        ---------- ---------- -----   [505]
D._c._fosteri           T..T.C.A.C ........A. ....A   [505]
D._c._forresteri        T..C.C.A.C ........A. ....A   [505]
D._singhi               C..CAC..G. ..C.....A. ....A   [505]
D._thompsoni U96424     ---------- ---------- -----   [505]
D._thompsoni U96425     T....C.... ..C.....A. ....A   [505]
D._collinsi             T....C.... ..C.....A. ....A   [505]
D._adamsae U96427       C..A.CT... ..C.....A. ....A   [505]
D._adamsae U96428       ---------- ---------- -----   [505]
D._wellsi               ---------- ---------- -----   [505]
D._wraggi               ---------- ---------- -----   [505]
D._simberloffi          ---------- ---------- -----   [505]
D. vauxi                T..T.CG.AA ........G. .A..G   [505]
D._cypsiurus            C..T.CT... ........G. ....G   [505]
D._hirundinis           T....CT.CC ..C.....A. .A..A   [505]
```

FIG. 4.—*Continued*

carried out using programs written by RDMP. The numbers of synonymous and nonsynonymous substitutions were computed using Li's (1993) method as implemented in Andrey Zarhkikh's program MATDISLI.

Initial investigations of phylogenetic signal in the data were made using the program Spectrum (Charleston, in preparation; available on the Internet from http://taxonomy.zoology.gla.ac.uk/~mac/spectrum/spectrum.html, which implements spectral analysis using the Hadamard transform (Hendy and Penny, 1993). A bipartition spectrum shows the distribution of support for each of the possible "splits" in a data set, where a split is any bipartition of the set of sequences. For example, given the sequences {A, B, C, D, E}, two of the possible splits are {{A, B}, {C, D, E}} and {{A},{B, C, D, E}}. For n sequences there are 2^{n-1} possible splits. We follow Hendy and Penny's (1993) convention of referring to each split by the decimal equivalent of the binary number obtained when the split is represented as a string of zeros and ones. To illustrate, the corresponding binary number for the split {{A, B},{C, D, E}} is $00011_{\text{base 2}} = 3_{\text{base 10}}$, and for the split {{A, C},{B, D, E}} it is $00101_{\text{base 2}} = 5_{\text{base 10}}$.

The support for a split is a function of the number of character state changes that correspond to that split. If the data correspond exactly to a single fully resolved

(binary) tree then only the $(2n - 3)$ splits corresponding to the edges (= branches) of that tree will have positive support. Of these splits, n represent the terminal sequences (here called "pendant edges") and hence are not informative about relationships among the sequences. The remaining $(n - 3)$ splits represent the internal edges of the tree and will be referred to as internal splits. If the data contain conflicting signals (for example, caused by homoplasy, where the same characters have evolved in different lineages) then more than $(n - 3)$ internal splits will have positive support and hence the data suggest more than one tree. At least some of these splits will be mutually incompatible, as there is no single tree that can accommodate more than $(n - 3)$ splits. Plotting the distribution of support for each split, together with the sum of support for all the other splits with which it is incompatible (that split's "conflict"), gives a visual display of the phylogenetic signal in the data.

The program Spectrum cannot compute spectra directly from four-state sequence data. Rather, the data must first be transformed into binary data, such as purines and pyrimidines (although an "average spectrum" can be computed from all seven possible mappings of four-state nucleotide data onto two-state characters). Alternatively, the program can compute spectra from distance matrices, and because of difficulties in handling missing data, this is the approach used here. Once a spectrum has been computed, various tree selection criteria can be applied to choose a tree that in some sense is best supported by the spectrum. In this study we used the "closest tree" (Hendy, 1991), which is the tree whose expected spectrum is closest to the observed spectrum.

Trees were also constructed from the data using maximum parsimony, maximum likelihood, and neighbor-joining, using the programs PAUP 3.1.1 (Swofford, 1993), fastDNAml (Olsen *et al.*, 1994), and the PHYLIP programs DNADIST, SEQBOOT, and NEIGHBOR (Felsenstein, 1993). COMPONENT (Page, 1993a) was used to compare morphological and molecular trees for the lice using partition (d_s) and quartet (d_q) measures of tree similarity (Steel and Penny, 1993). The probability of obtaining the observed values of these measures was computed by comparing 1000 pairs of random trees. TreeView (Page, 1996b) was used to prepare illustrations of trees. Maximum likelihood estimates of nucleotide sequence divergence in host and parasites were obtained using the PHYLIP programs DNAML and DNAMLK. The presence of a molecular clock was tested for using a likelihood difference test under the null hypothesis that twice the difference in log likelihoods, Δ, for clock and nonclock models is asymptotically distributed as χ^2 with $n - 2$ *df*, where n is the number of taxa in the tree (Felsenstein, 1993). Host and parasite phylogenies were compared using the program TreeMap (Page, 1994b). The probability of obtaining an observed number of cospeciation events was determined by comparing the actual host tree with 100 random parasite trees generated using a Markovian model (Harding, 1971). The correlation between host and parasite divergence was tested using the randomisation test described in Page (1996a).

RESULTS

Sequence Characteristics

We sequenced a 505-bp fragment of louse cytochrome *b* (Fig. 4) corresponding to positions 11323–11824 in the *Apis mellifera* mitochondrial genome (Crozier and Crozier, 1993). This fragment includes the entire 406-bp region of the host cytochrome *b* gene sequenced earlier (Lee *et al.*, 1996). Figure 5 shows the inferred cytochrome *b* amino acid sequence for the louse *Dennyus hirundinis* aligned with other insects, its swift host (*Ap. apus*), and the chicken (*Gallus gallus*). We are confident that these are louse sequences because no putative louse sequence was the same as that obtained from any bird sequenced in our laboratory and because the sequences grouped with other insect cytochrome *b* sequences in evolutionary trees we constructed.

The louse sequences show the A + T bias typical of other insect mitochondrial genomes. This bias is most pronounced at the third codon position, where 83% of the bases are either A or T (Table 2). The overall A + T composition is 69%, which places it in the middle of the range for exopterygotan insects (true bugs, lice, and their relatives) (Jermiin and Crozier, 1994). Figure 6 shows the proportion of transitions in all pairwise comparisons of the louse cyt *b* sequences. In comparisons between the *distinctus* and *thompsoni* species-groups, and between the two subgenera *Collodennyus* and *Dennyus* (ingroup and outgroup, respectively), transitions comprised some 40% of the total observed sequence difference. Comparisons between less divergent (<15% sequence difference) sequences typically showed a higher proportion of transitions, with the most similar sequences showing a wide scatter of values. The general pattern of the proportion of transitions approaching a maximum of ~40% of observed substitutions in sequences more than 20% divergent (Fig. 6) was also found by Liu and Beckenbach (1992) for insect mitochondrial COII sequences.

Phylogenetic Signal

The size of the spectrum for 20 sequences precluded spectral analysis of all the sequences (the program Spectrum is limited to 18 sequences or fewer), so we deleted 4 sequences that represented duplicates of other sequences and computed spectra for the remaining 16 sequences. The sequences deleted (U96416, U96424, U96418, and U96428) were selected because they were less complete than other sequences from the same taxa.

The spectrum for the subset of 16 sequences is shown in Fig. 7a, and the corresponding closest tree is shown

```
Dennyus    :  ------------------------------------------------------  :    -
Apis       :  MKKFMNFFSSNEFLKMIMS-TIYLPTPVNINYMWNFGSILGIFLMIQIISGF  :   51
Drosophila:   --MHKPLRNSHPLFKIANNALVDLPAPINISSWWNFGSLLGLCLIIQILTGL  :   50
Locusta    :  --MNKPLRIKHPMIKIINNSLNDLPAPTNISMWWNLGSLLGMCLMIQIVTGL  :   50
Apus       :  ------------------------------------------------------  :    -
Gallus     :  --MAPNIRKSHPLLKMINNSLIDLPAPSNISAWWNFGSLLAVCLMTQILTGL  :   50

Dennyus    :  ------------------------------------------------------  :    -
Apis       :  ILSMHYCPNIDIAFWSITNIMKDMNSGWLFRLIHMNGASFYFLMMYIHISRN  :  103
Drosophila:   FLAMHYTADVNLAFYSVNHICRDVNYGWLLRTLHANGASFFFICIYLHIGRG  :  102
Locusta    :  FLTMHYTPNIEMAFSSVVHICRDVNNGWLIRTLHANGASMFFICMYLHVGRG  :  102
Apus       :  ------------------------------------------------------  :    -
Gallus     :  LLAMHYTADTSLAFSSVAHTCRNVQYGWLIRNLHANGASFFFICIFLHIGRG  :  102

Dennyus    :  ---KSYLKTPLTWMSGVALYLLFMGVAFLGYVLPWGQMSYWGGTVITNLISA  :   49
Apis       :  LFYC..KLNN-V.GI .M.L..S.AA.................A.........  :  154
Drosophila:   IYYG...F..-...V..I.LF.V..T.................A.........  :  153
Locusta    :  IYYG...YMN-.....TI.LF.V.AT................A.........  :  153
Apus       :  ----------------------------------.....A......F..  :   16
Gallus     :  LYYG...YKE-..N...I.L.TL.AT................A......F..  :  153

Dennyus    :  VPYFGQSLVEWIWGGFSVSGPTLTRFFTLHYFLPLLSVAFILVHLTFLHKTG  :  101
Apis       :  ...I.D...L........NNA..N.......I....I.FM.....FA..L..  :  206
Drosophila:   ...L.MD.........A.DNA.......F..I..F.V..MT....L...Q..  :  205
Locusta    :  ...I.TD.........A.DNA..N....F..V..F.V..M.....F...Q..  :  205
Apus       :  ...I.......A......GN.......A...L..F.IAGLT........E..  :   68
Gallus     :  ...I.H.....A......DN.......A...L..FAIAGIT........E..  :  205

Dennyus    :  SSNPLGLSKNSDKVFFHPFFSYKDFLGFSFFGLLLIFFSMKYPNLFLDADNY  :  153
Apis       :  ......SNF.NY..S......I..L...YIILF.F..INFQ..YHLG.P...  :  258
Drosophila:   .N.....NS.I...P.........I...IVMIF...SLV.IS...LG.P...  :  257
Locusta    :  .N.....NS.I...P.........M.T.IILM....MLC.ID.Y.LG.P...  :  257
Apus       :  .N.....VS.C...P.....AT..P...IIMFTP..TLA.FS...LGGPE..  :  120
Gallus     :  .N......S.....P.........I..L.LMLTPF.TLA.FS...LG.PE..  :  257

Dennyus    :  SVANPMTTPAHIQPE----------------------------------------  :  168
Apis       :  K.....N..T..K..WYFLFAYSILRAIPNKLGGVIGLVMSILILYIMIFYN  :  310
Drosophila:   IP....V........WYFLFAYAILRSIPNKLGGVIALVLSIAILMILPFYN  :  309
Locusta    :  VP....V..I.....WYFLFAYAILRSIPNKLGGVIALVMSISILMIMPFYN  :  309
Apus       :  .P....V..P..K..----------------------------------------  :  135
Gallus     :  .P....V..P..K..WYFLFAYAILRSIPNKLGGVLALAASVLILFLIPFLH  :  309

Dennyus    :  ------------------------------------------------------  :    -
Apis       :  -NKMMNNKFNMLNKIYYWMFINNFILLTWLGKQLIEYPFTNINMLFTTTYFL  :  361
Drosophila:   LSKFRGIQFYPINQILFWSMLVTVILLTWIGARPVEEPYVLIGQILTIIYFL  :  361
Locusta    :  KTKFRGNQFYPMNQIMFWIMVIVICLLTWIGKRPVEEPYIMTGQILTIIYFS  :  361
Apus       :  ------------------------------------------------------  :    -
Gallus     :  KSKQRTMTFRPLSQTLFWLLVANLLILTWIGSQPVEHPFIIIGQMASLSYFT  :  361

Dennyus    :  ----------------------  :    -
Apis       :  YFFLN-FYLSKLWDNLIWNSPLN  :  383
Drosophila:   YYLIN-PLVTKWWDNLLN-----  :  378
Locusta    :  YFLFN-VHIAKMWDTLIKT----  :  379
Apus       :  ----------------------  :    -
Gallus     :  ILLILFPTIGTLENKMLNY----  :  380
```

FIG. 5. Inferred and aligned amino acid sequences of cytochrome *b* from the louse *Dennyus hirundinis* (this study), its avian host *Apus apus* (Lee *et al.*, 1996, GenBank Accession number U49981), the honey bee *Apis mellifera* (Crozier and Crozier, 1992, M87052), the fruitfly *Drosophila yakuba* (Clary and Wolstenholme, 1985, X00563), the migratory locust *Locusta migratoria* (Flook *et al.*, 1995, X80245), and the chicken *Gallus gallus* (Kornegay *et al.*, 1993, L08376). The sequences from *Dennyus* and *Apus* are incomplete. Sequence differences are given in relation to *Dennyus*. Identical amino acids are indicated by dots; hyphens denote gaps inserted to improve alignment.

in Fig. 7b. The spectrum shows that much of the evolutionary change in *Dennyus* cytochrome *b* is confined to terminal branches ("pendant edges") in the tree and hence is uninformative about phylogenetic relationships among the lice. By comparison, the internal splits have less support; however, several splits are relatively well supported and have little or only moderate conflict. The monophyly of the subgenus *Collodennyus* (split 8191) with respect to the subgenus *Dennyus* has moderate support, although there is weaker support (indi-

TABLE 2

Base Composition of *Dennyus* Louse Cytochrome *b* Sequences (Mean ± SE)

Base	First	Second	Third	Total
A	25.4 ± 0.77	18.5 ± 0.36	34.0 ± 0.76	26.0 ± 0.47
C	15.9 ± 0.50	20.8 ± 0.28	10.3 ± 1.06	15.7 ± 0.46
G	22.0 ± 0.50	17.6 ± 0.59	6.9 ± 1.04	15.4 ± 0.56
T	36.8 ± 1.16	43.2 ± 0.61	48.8 ± 1.72	42.9 ± 1.07

cated in Fig. 7a by the shaded bar below the *X*-axis) for placing one or more of the outgroups on the branches leading to one or other of the two species-groups within *Collodennyus*. The splits corresponding to these two subgroups of *Collodennyus* (splits 127 and 8064) are well supported, as are most splits within the *thompsoni* species-group. Within the *distinctus* species-group there is good support for splits uniting the two Indian Ocean subspecies of *D. carljonesi* (split 48) and the two subspecies of *D. distinctus* (split 3). Support for the remaining splits within the *distinctus* group (12, 67 and 79) is weak; there are one or more splits not in the closest tree (i.e., incompatible with that tree) which have more support than these splits (Fig. 7a).

Tree Construction

The results of parsimony, maximum likelihood, and neighbor joining are shown in Fig. 8. All three methods produced trees that clearly separate the *distinctus* and *thompsoni* species-groups, and within the latter group the *thompsoni* and *francicus* species-subgroups are also clearly monophyletic. All methods agree on relationships among the *thompsoni* species group, save whether *D. collinsi* lies outside or within the two *D. thompsoni* sequences. The trees are less concordant concerning relationships among the *distinctus* species group lice. They all agree on pairing the two Indian Ocean subspecies of *D. carljonesi,* the two *D. distinctus* subspecies, and the sequences of *D. somadikartai* from different locations, but the relationships among these three sets of sequences and the remaining members of the *distinctus* group are unclear. The three pairs of sequences that all methods recover are supported by bootstrap values of 100%, whereas the bootstrap values for the other nodes in this clade are weaker. The minor discrepancies between the tree methods are not unexpected given the spectrum for these sequences (Fig. 7a), which indicates that the relevant splits in the *distinctus* species-group have weak support at best and have large values of conflict. Given weak, contradictory information, the three tree selection criteria used here have picked out different signals within the *distinctus* clade.

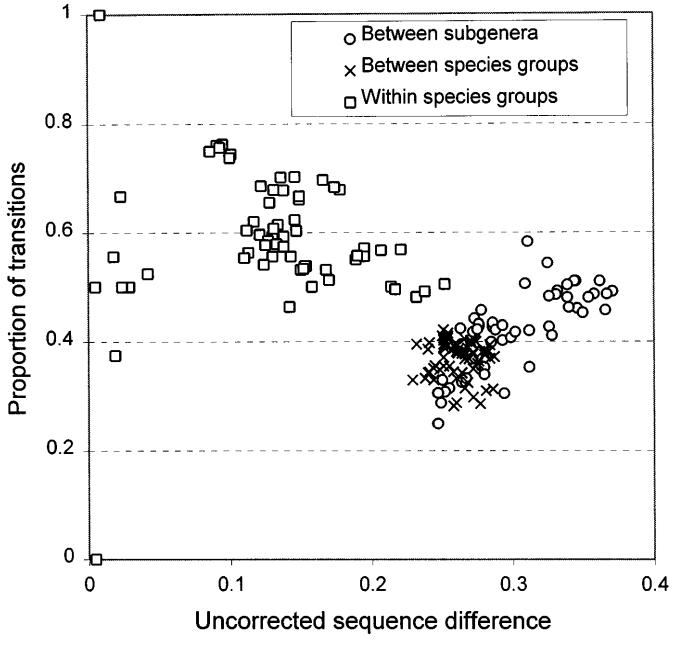


FIG. 6. Plot of the proportion of transitions against uncorrected sequence difference for all pairs of louse cytochrome *b* sequences. Comparisons between the two subgenera (*Dennyus*(*Collodennyus*) and *Dennyus*(*Dennyus*)), between the *distinctus* and *thompsoni* species-groups, and within the two species groups are distinguished (see Fig. 3).

Phylogenetic Conclusions

The trees we obtained from the cytochrome *b* sequences are highly concordant with the dendrograms resulting from cluster analyses of morphometric data for male and female lice (Clayton *et al.,* 1996). If the molecular and morphological trees are pruned to the 15 taxa they have in common, the dendrogram shown in Fig. 3 is much more similar (by both the partition and the quartet measures of tree similarity) to the three molecular trees shown in Fig. 8 than would be expected if the two sets of trees were chosen at random ($d_s = 10$, $P < 0.001$; $d_q = 142–150$, $P < 0.001$). However, it is important to remember that generation of the morphological and molecular trees was not completely independent. As discussed above, the identity of three sequences we obtained (*D. d. distinctus, D. thompsoni,* and *D. simberloffi*) was inferred by comparing their placement in the molecular trees with the relationships predicted by morphometric data. For example, sequences U96424 and U96425 were identified as *D. thompsoni* because they grouped with the *D. collinsi* sequence, which is the expected placement for this taxon based on the phenogram shown in Fig. 3. However, pruning *D. d. distinctus, D. thompsoni,* and *D. simberloffi* from the molecular and morphological trees did not significantly alter the degree of congruence between the molecular and morphological trees.

Considering the relative lack of support for relationships within the *distinctus* species group, compared to those within the *thompsoni* clade, we adopt a conservative phylogenetic hypothesis of relationships among swiftlet lice for comparison with swiftlet relationships (Fig. 9). This tree shows the basal split of the subgenus *Collodennyus* into the *distinctus* and *thompsoni* species

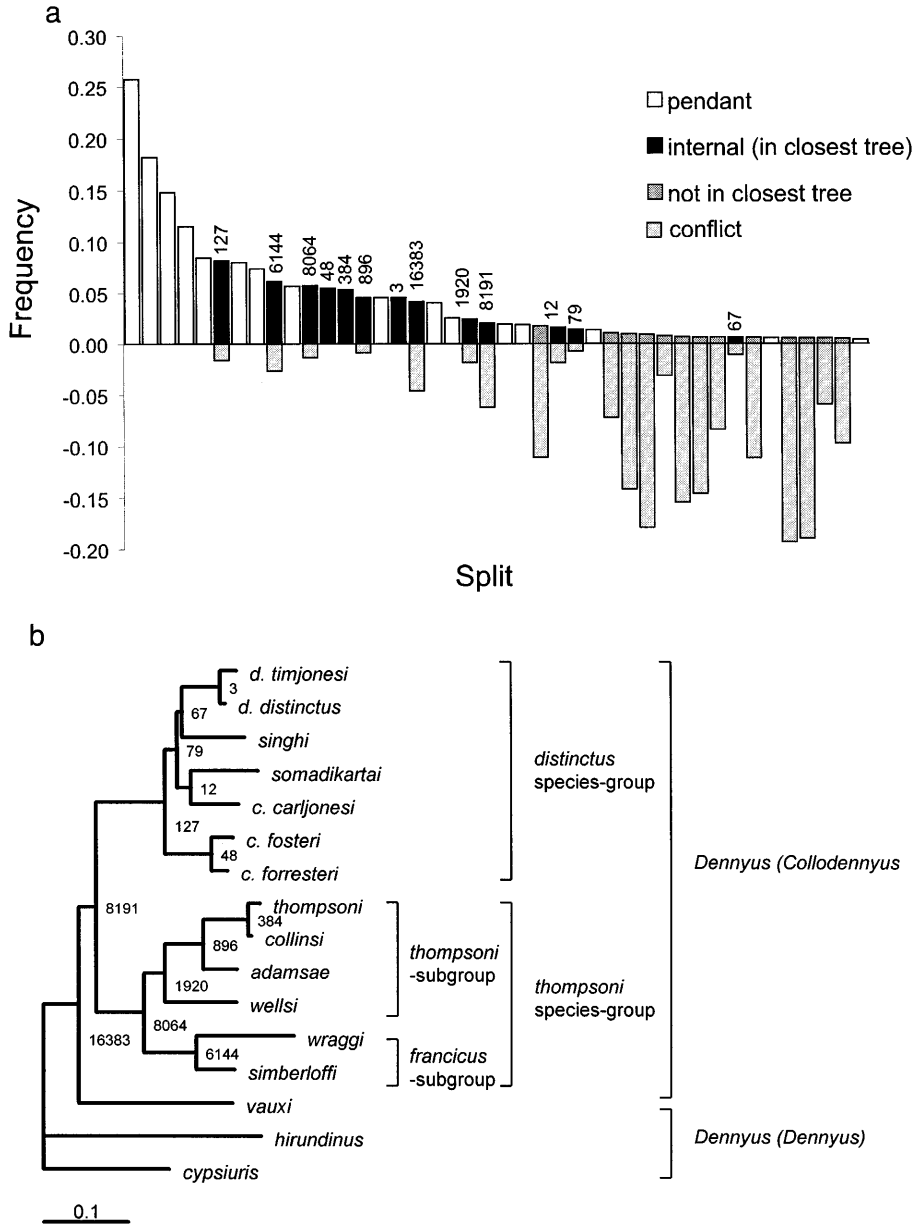


FIG. 7. (a) Support/conflict spectrum and (b) closest tree for maximum likelihood distances for louse cytochrome *b* sequences. Splits are numbered using Hendy and Penny's (1993) scheme. In the spectrum splits are ordered from left to right by their support (in units of expected number of substitutions per site); bars above the *X*-axis represent support for each split; bars below each split represent the sum of support for all conflicting splits. Each split is distinguished by whether it is a pendant edge (i.e., a terminal taxon), an internal edge in the closest tree (labeled in (b)), or a split that is not in the closest tree. Conflict values are normalized following Lento *et al.* (1995). The scale bar below the closest tree is in units of expected number of substitutions per site.

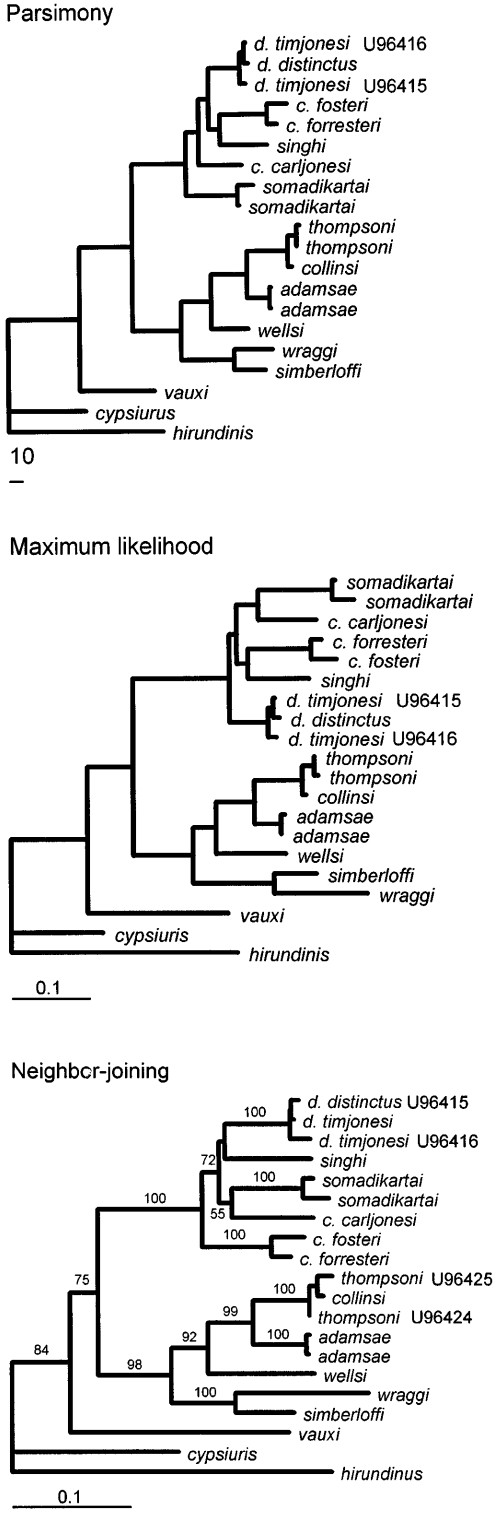
groups, and relationships among lice in the *thompsoni* group are fully resolved. The weak phylogenetic signal for the *distinctus* group sequences limits our phylogenetic conclusions to the monophyly of the two subspecies of *D. distinctus* and the monophyly of the two Indian Ocean lice, *D. carljonesi forresteri* and *D. c. fosteri.*

Rates of Louse Evolution Relative to That of Other Insects

Following Crozier and colleagues (Crozier and Crozier, 1992; Crozier *et al.,* 1989) we computed the number of unambiguous amino acid replacements for the shared portion of cytochrome *b* by constructing unrooted trees for subsets of four species ("quartets"). Each quartet included *Dennyus hirundinis,* the swift *Ap. apus,* the mouse *Mus,* and one other insect. By comparison with *Drosophila* (Fig. 10a), *Dennyus* cytochrome *b* is highly divergent, having accumulated up to 4.5 times as many amino acid replacements. Crozier and Crozier (1992) found a similar disparity in divergence between hymenopterans and *Drosophila;* Fig. 10b shows that the degree of divergence in *Dennyus*

and *Apis* cytochrome *b* is more comparable (ratio of divergence between *Dennyus* and *Apis* ~1.5). When compared to *Locusta*, *Dennyus* shows a twofold greater rate of divergence and is nearly three times more divergent than *Anopheles*.

The only other protein coding gene sequences available for lice are the cytochrome oxidase I (COI) data obtained by Hafner *et al.* (1994) for pocket gopher lice, which belong to a different suborder (Ischnocera) than swiftlet lice (Amblycera) and have mammalian hosts. Quartets involving *Geomydoecus* show the same pattern as *Dennyus*, and gopher lice are more (~3.75 times) divergent than *Drosophila* (Fig. 10c), *Locusta* (2.7) and *Anopheles* (5.3) and twice as divergent as *Apis* (Fig. 10d). Although the swiftlet and gopher louse protein sequences are short, they suggest that protein coding genes of louse mtDNA may be among the most divergent of all insects.



Host–Parasite Cospeciation

For an initial assessment of relative evolutionary divergence between louse and bird proteins we constructed quartets for pairs of *Dennyus* lice and their hosts (Fig. 11). Pairs of louse taxa were invariably more divergent than pairs of the corresponding hosts. In order to discuss rates of evolution (as opposed to amounts of divergence alone) we need to be able to ascertain the relative ages of the lice and their hosts. Swifts have a poor fossil record (Harrison, 1984) and lice have none whatsoever, so calibrating the ages of these two taxa rests on identifying cospeciation events in the two clades. As pointed out above, it is not enough simply to compare divergence in pairs of hosts and parasites (Fig. 2). Only if the host birds and their parasitic lice have strictly cospeciated will differences in divergence directly reflect differences in rates of evolution. If the lice are older than their swift and swiftlet hosts, then the great divergence among lice may reflect their greater antiquity; alternatively, if the lice have not cospeciated with their hosts but instead have recently colonized them, then the louse lineages are younger than their hosts and are evolving at a much greater pace.

The limited taxonomic sampling and poor resolution of relationships among the *distinctus* taxa makes it difficult to interpret their history with respect to swift phylogeny. Certainly, methods such as that of Page (1994b) which require fully resolved phylogenies cannot be applied to our bird and louse trees shown in Fig. 9. However, these methods can be applied to a subset of the taxa.

A cospeciation event is a pairing of a node in the parasite tree with a node in the host tree [in Page's (1994a) terminology this pairing is a "map" between the

FIG. 8. Trees computed for the cytochrome *b* nucleotide sequences shown in Fig. 4. (a) The single most parsimonious tree (811 steps, consistency index, ci = 0.61, retention index, ri = 0.69), (b) maximum likelihood tree (ln $L = -4259.39$), (c) neighbor-joining tree computed from maximum likelihood distances with nodes labeled by their percentage of occurrence among 1000 bootstrap trees. Sequences from the same louse taxon that did not group together are distinguished by their GenBank Accession numbers (see Table 1). Scale bar in (a) represents 10 nucleotide substitutions; in (b) and (c) the bar represents 0.1 substitutions per site.

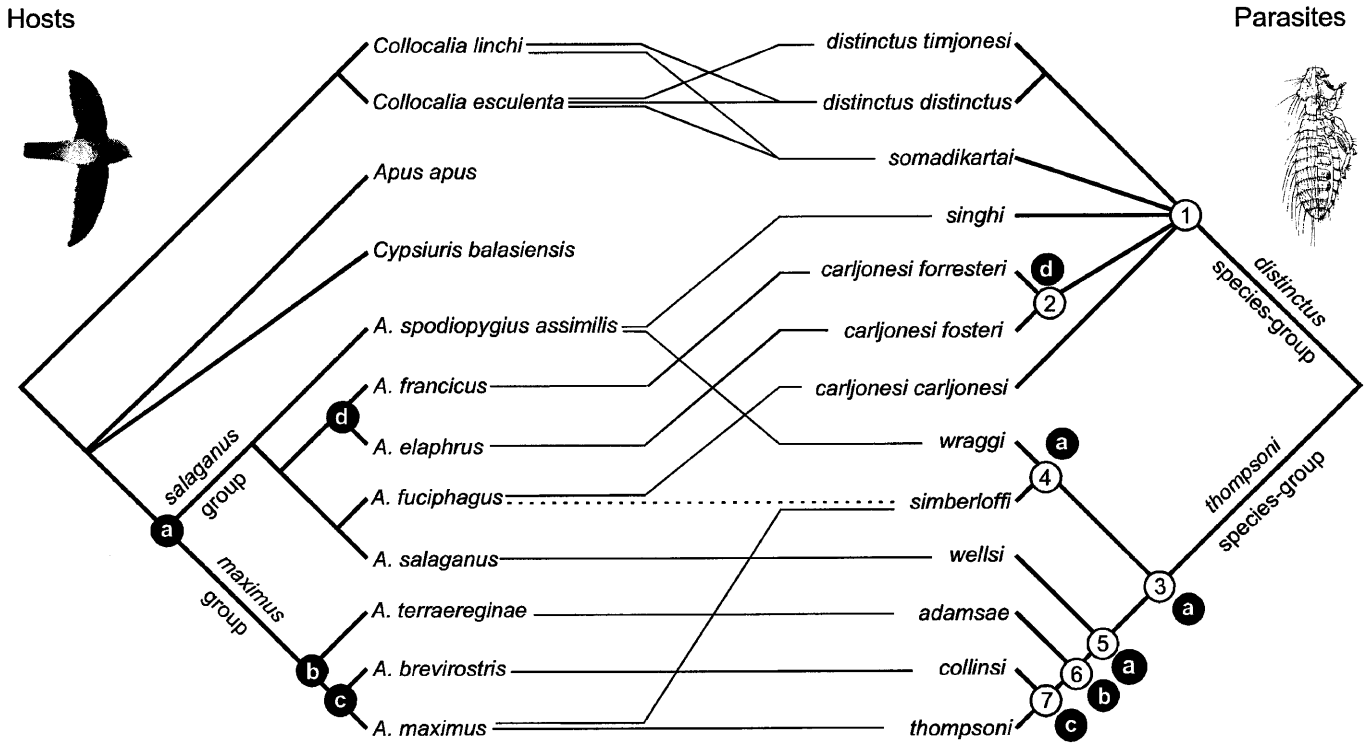


FIG. 9. Cladograms for *Dennyus* (*Collodennyus*) lice and their hosts. Numbered nodes in the louse tree are discussed in the text. For nodes 2–7 corresponding nodes in the host cladogram are indicated by the letters a–d. The phylogeny of swifts and swiftlets is from Lee *et al.* (1996). *A* is the genus *Aerodramus*. Although the *D. simberloffi* louse we sequenced was obtained from an individual of *Aerodramus fuciphagus* (indicated by the dashed line), the primary host of this louse is *A. maximus* (Clayton *et al.*, 1996).

two nodes]. A simple rule for identifying putative cospeciation events is to associate a parasite node with the host node that is the most common ancestor of all the hosts infested by descendants of the parasite node. For example, the lice *D. collinsi* and *D. thompsoni* are hosted by *Aerodramus brevirostris* and *A. maximus* (Fig. 9). The common ancestor of the two lice, represented by node 7 in Fig. 9, can be mapped onto the common ancestor of their hosts, which is node c. Likewise, node 6 in the louse cladogram corresponds to node b in the bird tree, as node b is the most recent common ancestral host of the three lice that descend from node 6. Hence these three lice (*adamsae, collinsi,* and *thompsoni*) appear to have cospeciated with swiftlets belonging to the "*maximus*" group of *Aerodramus.* Applying the same logic to node 5 links that node with node a in the host tree, implying that if members of the louse clade rooted at node 5 have cospeciated with their hosts then *D. wellsi* is the sole extant representative of that clade on the "*salanganus*" group.

The two louse species of the *francicus* species-subgroup for which we have sequences, *D. simberloffi* and *D. wraggi,* were obtained from birds belonging to the *salanganus* group. However, although the *D. simberloffi* sequence was obtained from a louse hosted by an individual of *Aerodramus fuciphagus* in Gomantong Caves, the primary host of this louse appears to be *A. maximus.* These two birds nest in close proximity in Gomantong Caves, and it appears that this proximity has provided opportunities for *D. simberloffi* individuals to accidentally infest hosts other than *A. maximus* (Tompkins and Clayton, in preparation). Hence, the ancestor of *D. simberloffi* and *D. wraggi* (node 4) probably corresponds to node a in the bird phylogeny. As a consequence node 3 represents a "duplication"—speciation of the ancestor of the *thompsoni* group prior to any speciation in the ancestor of the hosts of that group.

Relationships within the *distinctus* species group are poorly resolved, as represented by the basal polytomy (node 1) in Fig. 9. The only node that can be linked readily with a corresponding node in the host phylogeny is node 2, the ancestor of the two Indian Ocean subspecies of *D. carljonesi.* Their hosts, *Aerodramus elaphrus* and *A. francicus,* are also sister taxa; hence node 2 is associated with node d.

Figure 12a shows the subtree for the lice that results after removing all nodes other than those that we can satisfactorily map onto nodes in the host tree, together with the phylogeny of the hosts of those lice. For these subtrees the degree of match is highly significant ($P < 0.01$; based on 100 randomizations).

Comparing Rates of Sequence Divergence

In order to compare rates of sequence divergence in cospeciating hosts and parasites we need an approxi-

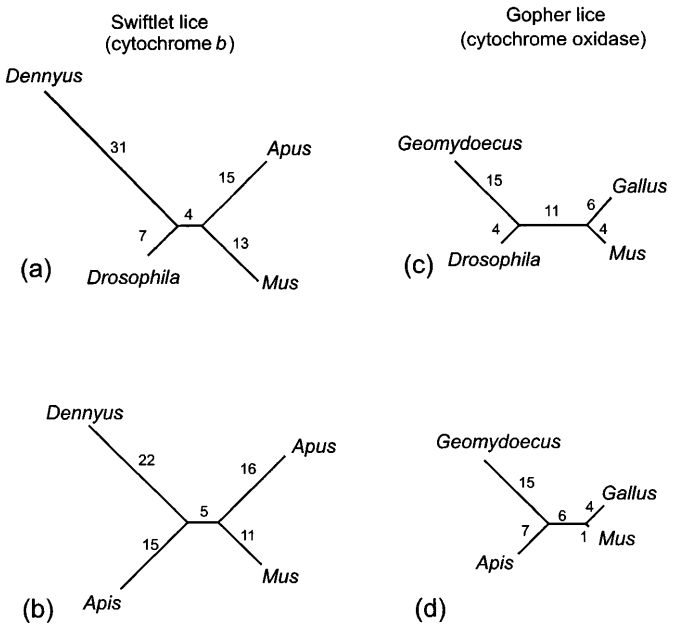


FIG. 10. Unrooted trees for quartets of cytochrome *b* (cyt *b*) and cytochrome oxidase I (COI) sequences. Each edge is labeled with the number of amino acid replacements that can be assigned unambiguously to that edge using parsimony. The louse cyt *b* (*Dennyus hirundinis*) sequence has diverged ~4.5 times more from a common ancestor than has that of *Drosophila yakuba* (a), whereas (b) the ratio of louse and honeybee (*Apis mellifera*) divergence is ~1.5. COI from the pocket gopher louse *Geomydoecus panamensis* (Accession number L32676) has diverged ~3.75 times more from a common ancestor than has that of *Drosophila* (c) and about twice as much as COI from the honeybee (d).

mately constant rate of evolution within each clade, that is, a molecular clock within each. The two clocks may tick at different rates and so be local clocks, but they need to be present, otherwise it is difficult to separate rate variation within lineages from that between hosts and parasites (Page, 1996a). To test for molecular clocks we computed maximum likelihood branch lengths for the host and parasite subtrees shown in Fig. 12a, both with and without imposing a molecular clock constraint (all codon positions were equally weighted, and the transition:transversion ratio was 2.0 for both birds and lice). Branch lengths were computed for all sites and for the third codon positions alone. For all positions considered together we could not reject the clock hypothesis for either birds (log likelihood difference $-2\Delta = 5.82$, $P = 0.32$) or lice ($-2\Delta = 12.19$, $P = 0.06$), whereas only in lice are third codons evolving at a constant rate. Hence we have restricted the comparison to all sites.

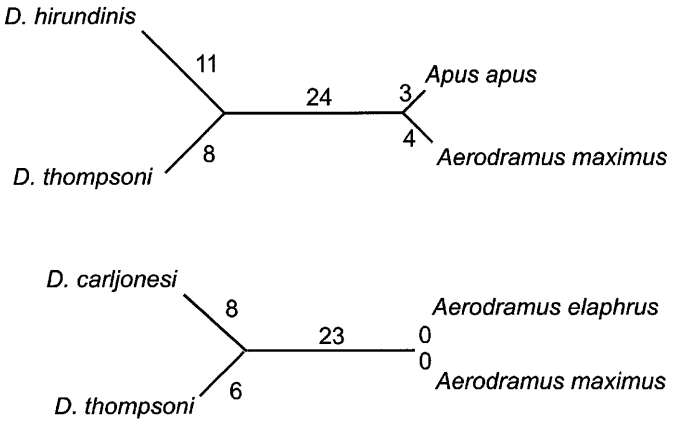


FIG. 11. Quartets for pairs of cytochrome *b* sequences from *Dennyus* lice and their hosts (bird sequences from Lee *et al.*, 1996). Branches leading to the lice show more unambiguous amino acid replacements (numbers on each edge) than those leading to their hosts.

Because the subtrees for birds and lice are not identical (Fig. 12a) and could not be made identical without drastic pruning we did not apply Hulsenbeck *et al.*'s (1997) maximum likelihood test for equal rates among parasites and hosts. Instead we plotted parasite divergence against host divergence for each pair of cospeciation events (Table 3; Fig. 12b). The equation for the line fitted to these points using reduced major axis regression is $y = 2.56x - 0.01$, indicating that *Dennyus* louse mitochondrial cytochrome *b* is acquiring nucleotide substitutions more than twice as rapidly as the homologous host gene. The correlation between bird and louse divergence is significant, based on the randomization test described in Page (1996a, pp. 159–160) ($r = 0.895$, $P = 0.04$, based on 100 randomizations).

DISCUSSION

The high degree of congruence between morphological and molecular data sets suggests that the trees presented in this paper are reasonable estimates of the true phylogeny of *Dennyus* lice. Unfortunately, we had little success resolving relationships among the *distinctus* species-group lice, as reflected in the low values of support and large conflict values for most splits within this clade (e.g., splits 12, 67, and 79 in Fig. 7a). Obtaining additional sequence data for the *distinctus* taxa we sampled may help resolve this polytomy in the future. Another consideration is that, to date, we have collected molecular data from only half the members of this clade. The addition of further taxa may also help clarify relationships within this group. In contrast, the *thompsoni* species-group is both well sampled and well resolved.

Contradictory evidence at the base of the louse tree (see splits 8191 and 16383 in Fig. 7a) was resolved by all tree building methods in favor of a monophyletic *Collodennyus* (Fig. 8). Less contradictory information about relationships among the putative outgroups and the two species groups of *Collodennyus* might be obtained from a more slowly evolving gene such as 12S rRNA. The large amount of sequence divergence between the subgenera *Dennyus* and *Collodennyus* (Fig.

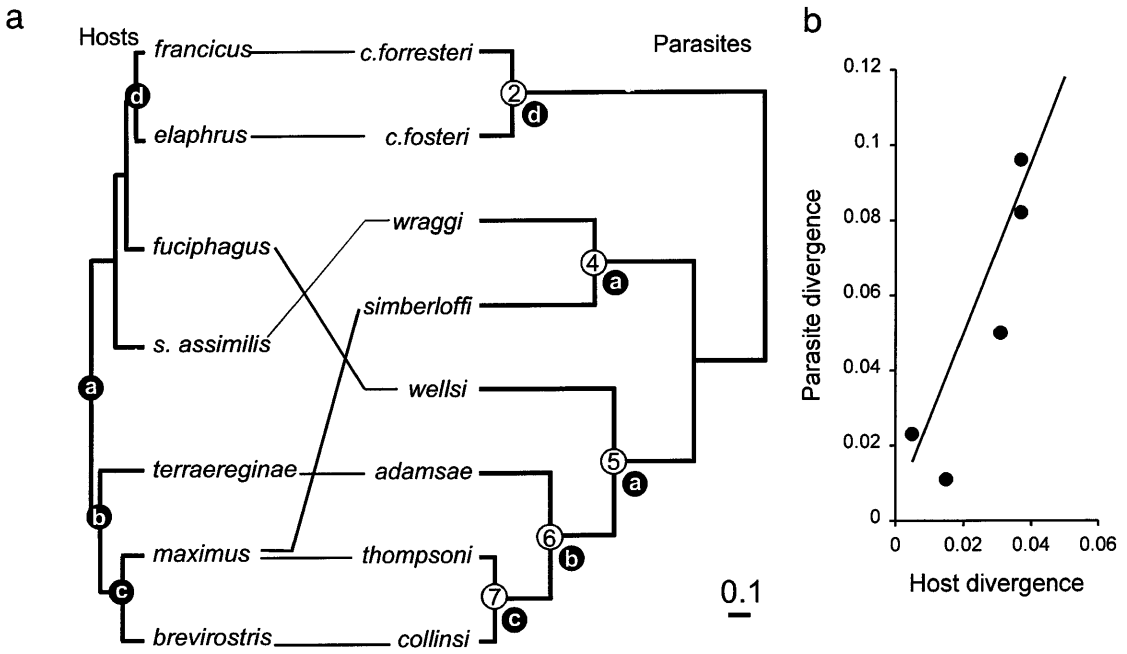


FIG. 12. (a) Subtrees of cospeciating birds and lice. These trees result from pruning off all the louse lineages in Fig. 9 except those that can be readily mapped onto nodes in the host tree. The resulting subtrees are drawn with branch lengths proportional to the expected number of substitutions per site for the same region of cytochrome *b* and are drawn to the same scale. Branch lengths were computed over all sites using maximum likelihood with a molecular clock imposed. Nodes are labeled as in Fig. 9. The thin lines connect each louse sequence with the host from which each louse was collected (except *Dennyus simberloffi*, which is primarily found on *Aerodramus maximus*, although our sequence was obtained from a louse collected from *A. fuciphagus;* see text). The scale bar is in units of expected number of substitutions per site. (b) Parasite divergence plotted against host divergence for the five cospeciation events identified in (a).

6) suggests that cytochrome *b* may be approaching its limits of resolution at this phylogenetic depth.

In contrast to their great morphological conservatism (Clayton *et al.*, 1996), *Dennyus* lice have among the most divergent insect cytochrome *b* reported, although there are currently few insect sequences of this gene available for comparison. The disparity in relative divergence between, for example, *Drosophila* and *Dennyus*, is further evidence for a wide range in rates of mitochondrial evolution among insects (Crozier and Crozier, 1992). Hence the difference in rate of evolution of avian and louse mtDNA cannot simply be attributed to an overall difference between rates of evolution in birds and insects. Gopher lice, the only other lice for which we have data from mitochondrial protein genes, show similar amounts of divergence (Fig. 10) to swiftlet lice. Given that gopher lice and swiftlet lice belong to different louse suborders, this raises the possibility that this pattern may be general among lice. Few other insects groups, with the exception of the Hymenoptera (wasps, ants, and bees), show comparable amounts of divergence.

Some of the apparent disparity in divergence between louse and swiftlet sequences appears to be due to the presence of multiple lineages of lice on the same birds. These lineages evidently diverged prior to the radiation of the swifts; hence these louse lineages are older than their hosts. Sequence divergence between randomly chosen representatives of these louse lineages will therefore reflect this antiquity. Such comparisons are analogous to those between paralogous genes where differences between such sequences (and any inference of time of divergence based on those differences) relate to earlier gene duplications rather than to the age of the organisms from which those sequences were obtained. In the same way, comparisons of se-

TABLE 3

Divergence in Expected Number of Nucleotide Substitutions per Site for Each Pair of Cospeciation Events in the Phylogenies Shown in Fig. 12a

Cospeciation event[a]	Divergence	
	Host	Parasite
a-4	0.037	0.082
a-5	0.037	0.096
b-6	0.031	0.050
c-7	0.015	0.011
d-2	0.005	0.023

For each pair of nodes the divergence is the "depth" of the node in the phylogeny, that is, the sum of branch lengths along the path from the node to any of its descendants.

[a] Host and parasite nodes labeled as in Fig. 12a.

quence divergence between "paralogous" lineages of lice will not be directly comparable to divergence among the homologous genes in the hosts; rather these deep divergences within the louse tree will relate to earlier events in the bird–louse association (Fig. 2). An example of this is the comparison between the lice *D. carljonesi* and *D. thompsoni* and their hosts (Fig. 11). If our interpretation of the history of this host–parasite assemblage is correct, these lice are paralogous. This emphasises the need to identify cospeciation events in host and parasite clades in order to accurately calibrate rates of evolution in the two groups; failure to do so may greatly exaggerate the apparent disparity in rates between the two groups of organisms. For those louse taxa for which we have well-resolved relationships there is a significant match with the phylogeny of their avian hosts. However, because of the lack of resolution of the *distinctus* species-group, we are unable to determine to what extent that clade has tracked their hosts over time, beyond the apparent cospeciation of the Indian Ocean subspecies of *D. carljonesi* and their hosts *A. elaphrus* and *A. francicus.* The co-occurrence of the same species of louse on unrelated hosts in Gomantong Caves in Indonesia (such as *D. simberloffi* discussed above) is evidence that swiftlet lice can disperse to foreign hosts, suggesting that they may have successfully colonized new hosts in the past.

A range of mechanisms has been proposed to account for differences in rates of mitochondrial DNA evolution among taxa, including generation time, metabolic rate, base composition, and population size (Rand, 1994). Difference in generation time between lice and their hosts has been offered as an explanation for the higher rate of substitution in pocket gopher lice and their hosts (Hafner *et al.,* 1994). However, the apparent correspondence between the magnitudes of the rate difference and generation length in these two organisms is weakened by subsequent analyses suggesting a less pronounced difference in evolutionary rate between gophers and lice (Huelsenbeck *et al.*, 1997; Page, 1996a). These reanalyses suggest that gopher lice are accumulating nucleotide substitutions only two to three times more rapidly than their mammalian hosts. Our analyses suggest that *Dennyus* lice show a similarly elevated rate compared to their avian hosts. By itself, generation time seems a poor explanation of variation among insects, given that *Drosophila* with its short generation time evolves more slowly than the relatively long-lived but highly divergent honeybee (Crozier *et al.*, 1989). Variation in base composition is also a poor predictor of variation in protein sequence divergence in insects (Jermiin and Crozier, 1994).

Dowton and Austin (1995) found an increase in the rate of mtDNA sequence evolution in wasps coincident with the adoption of a parasitic lifestyle. Such a lifestyle could increase the rate of sequence divergence, either though an increased frequency of founder events or through increased selection pressure due to a genetic arms race between host and parasite. While adaptive evolution may explain our data, we have no obvious reason to postulate increased selection on cytochrome *b* itself. Furthermore, the relationship between the number of synonymous and nonsynonymous substitutions appears similar in swiftlets and their lice, despite the much greater average pairwise divergence exhibited by the lice (Fig. 13). If the ratio between synonymous and nonsynonymous substitutions is a measure of selection (Li and Graur, 1991), the similar ratio in birds and lice suggests that any difference in amount of divergence between host and parasite genes is due to a difference in overall rate of substitution, rather than a change in selection regime. It would be interesting to obtain sequences from other mitochondrial genes and from nuclear genes of lice to see whether the elevated rate of evolution in cytochrome *b* applies to the genome as a whole. Sequences for a range of lice, together with data from the sister taxon of lice (Psocoptera) and other related clades, would help to establish whether the elevated rate of evolution in lice evolved at the same time they became ectoparasitic or whether it is a property of a larger clade to which lice belong.

An alternative explanation for the high rates of evolution in lice involves a combination of low louse effective population size and the restricted mode of transmission between hosts. For *D. hirundinis* on the swift *Ap. apus,* Lee and Clayton (1995) found a mean of

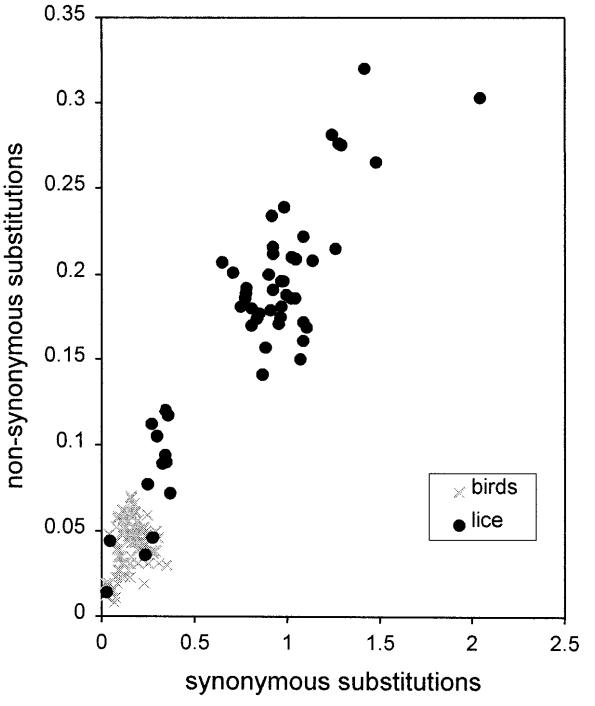


FIG. 13. Plot of nonsynonymous against synonymous substitutions for pairs of swift and swiftlet hosts and pairs of lice taxa from those hosts.

only two hatched lice per bird, with about nine eggs per bird. These lice are transmitted from parent birds to their nestlings (i.e., vertical transmission). This suggests that even though the total *Dennyus* louse populations are larger than the host populations (each bird having on average two lice), the transmission dynamics of the parasites may result in a lower effective population size for the lice. Nadler *et al.* (1990) found low levels of heterozygosity in gopher louse demes and differentiation among populations of lice resident on different individual hosts from the same host population. They suggested that the lice may undergo founder events with each initial infection of a juvenile gopher. If lice are predominantly transmitted from parent to offspring, and if the number of individuals that succeed in infecting the new host individual is small, then this succession of founder events could conceivably account for the accelerated rate of sequence evolution observed in both swiftlet and gopher lice (Ohta, 1972, 1987). DeSalle and Templeton (1988) have argued that founder events accelerated the rate of molecular evolution in some lineages of Hawaiian *Drosophila.* If louse population size and transmission dynamics influence rates of molecular evolution, it would be interesting to compare rates of evolution in louse taxa which vary in these parameters. For example, comparisons between lice found on colonial versus noncolonial species of host could be a promising direction for future research (Rózsa *et al.*, 1996).

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