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A HISTORY OF HOST ASSOCIATIONS AND EVOLUTIONARY DIVERSIFICATION FOR *OPHRAELLA* (COLEOPTERA: CHRYSOMELIDAE): NEW EVIDENCE FROM MITOCHONDRIAL DNA

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A rapidly growing supply of phylogenetic trees has fueled a burst of insights into the ways that phylogenetic data might inform our study of evolution (e.g., Lauder 1990; Wanntorp et al. 1990; Brooks and McLennan 1991; Harvey and Pagel 1991; Swofford and Maddison 1992). One area in which these approaches have proven particularly useful is the study of coevolution (Mitter and Brooks 1983; Brooks 1988; Miller 1991; Mitter et al. 1991; Brooks and McLennan 1993), the cladogenetic and selective influences that ecologically coupled taxa exert on each other through evolutionary time. Those who study the coevolution of herbivorous insects and their host plants have repeatedly noted both the relative host specificity of most herbivore species and the taxonomic conservatism that often characterizes the host affiliations of insect genera and families (Dethier 1954; Ehrlich and Raven 1964; Ward and Spalding 1993). Certain explanations for these patterns can be tested phylogenetically.

For example, if herbivore lineages remain strictly associated with their host species over sufficient periods of evolutionary time, vicariance events that isolate populations of the host will also isolate populations of the herbivore. To the extent that these events lead to the formation of new species, this combination of host loyalty and parallel cladogenesis (Benson et al. 1975; Mitter and Brooks 1983; Spencer 1988) can account for both host specificity and host conservatism. If this allopatric cospeciation scenario (Brooks 1979) holds, the phylogenies of host plant taxa and their insect parasites will be at least roughly concordant. Topological contradictions between these trees, however, suggest that herbivore lineages have switched their affinities from one plant group to another subsequent to the diversification of these plants (Jermy 1984; Brooks 1988). To date, most of the handful of pertinent studies (reviewed in Mitter and Farrell 1991) have found evidence for such host shifts, but a few notable exceptions are strongly consistent with the cospeciation model (Farrell and Mitter 1990; B. Farrell in prep.).

But if host shifts commonly occur, why do they involve the particular (and often related) plant species that they do, given that selection pressures favoring the use of locally common nonhosts must be omnipresent (Futuyma 1983)? Such conservatism suggests that the spectrum of likely host shifts may be limited by constraints on adaptively relevant genetic variation, perhaps because of the host associations of an herbivore lineage. For instance, the apparent role of plant chemistry in determining herbivore host affiliations (reviewed in Feeny 1992) might be largely explained by the minimally novel genetic variation required for shifts between chemically similar hosts.

We have adopted an historically informed approach to investigate the degree to which such genetic constraints can explain the host associations of the leaf beetle genus *Ophraella* (Futuyma 1992; Futuyma et al. 1993, 1994). These studies were initiated by optimizing host affiliations on the phylogeny of *Ophraella* (Maddison and Maddison 1992) in order to infer a history of host associations. *Ophraella* species with various histories of host use were then screened for genetic variation in performance on several hosts of their congeners to test the role that constraints on this variation might play in guiding the evolution of host shifts (Futuyma and Keese 1992; Futuyma et al. 1993, 1994, 1995). Futuyma et al. (1995) summarize and interpret the genetic data in the phylogenetic context reported in this paper. This research program thus represents a synthesis of phylogenetic and population biological approaches.

A phylogeny for these studies was first provided by the analysis of morphological and allozyme characters (Futuyma and McCafferty 1990, hereafter referred to as F&M). However, although F&M reported the resolution of *Ophraella* into three major clades, the relationships among the closely related species in the largest clade remained unclear. The importance of these unresolved relationships for interpreting the experimental work prompted the recent collection of rapidly evolving mitochondrial DNA (mtDNA) sequences. These data have been analyzed alone and in combination with the morphology and allozyme data to provide a more compelling estimate of *Ophraella* phylogeny. Here, we briefly report the results of this work, presented in full by Funk et al. (1995).

In this paper, we employ this new phylogenetic estimate to infer the history of *Ophraella* host associations at two taxonomic levels. We then evaluate our confidence in these results with reference to the ambiguities of character reconstruction, and discuss the insights and reinterpretations they provide about the history of host use. Unanticipated conclusions about the tempo and mode of *Ophraella* diversification are drawn from our genetic, phylogenetic, biogeographic, and host use data. In a companion paper, the new estimate of phylogeny is used to interpret the experimental studies on genetic constraints (Futuyma et al. 1995).

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TABLE 1. Geographical distributions and known host associations of species in this study. Abbreviations used in table 2 follow species' names. Abbreviations for states in the United States are used. Asteraceous tribes that include *Ophraella* hosts are abbreviated as follows: ANT, Anthemideae; AST, Astereae; EUP, Eupatorieae; HEL, Heliantheae. Further details on host records may be found in Futuyma (1990). Distributions are from LeSage 1986 and the personal records of D. Futuyma.

Species	Distribution	Host plants
Ophraella		
arctica LeSage (arc)	Arctic Canada	AST: *Solidago multiradiata*
artemisiae Futuyma (art)	W Tex., Ariz., Minn.	ANT: *Artemisia carruthii, A. ludoviciana*
bilineata (Kirby) (bil)	Great Plains of N U.S., S Canada	AST: *Chrysopsis villosa*
communa LeSage (com)	S Canada to S Mexico throughout	HEL: *Ambrosia artemisiifolia, A. psilostachya, Iva axillaris, Parthenium hysterophorus, Xanthium strumarium; Helianthus ciliaris*
conferta (LeConte) (con)	S Canada to N.C., E of Rocky Mts.	AST: *Solidago altissima* complex, *S. juncea, S. rugosa*
cribrata (LeConte) (cri)	S Canada; U.S. except SW	AST: *Solidago juncea, S. altissima, S. pinetorum, S. bicolor, S. nemoralis*
notata (Fabricius) (not)	E U.S.	EUP: *Eupatorium perfoliatum, E. maculatum, E. hyssopifolium, E. capillifolium*
notulata (Fabricius) (ntl)	Atlantic and Gulf coasts; scattered inland records	HEL: *Iva frutescens, I. annua*
nuda LeSage (nud)	SE Alberta	HEL: *Iva axillaris*
pilosa LeSage (pil)	U.S. and S Canada, E of Rocky Mts.	AST: *Aster macrophyllus, A. urophyllus, A. Lowrieanus, A. novae-angliae, A. cordifolius, A. paniculatus, Solidago bicolor, S. squarrosa*
sexvittata (LeConte) (sex)	SE U.S., N to N.C.	AST: *Solidago altissima, S. leavenworthii, S. gigantea*
slobodkini Futuyma (slb)	Fla., records from La.	HEL: *Ambrosia artemisiifolia*

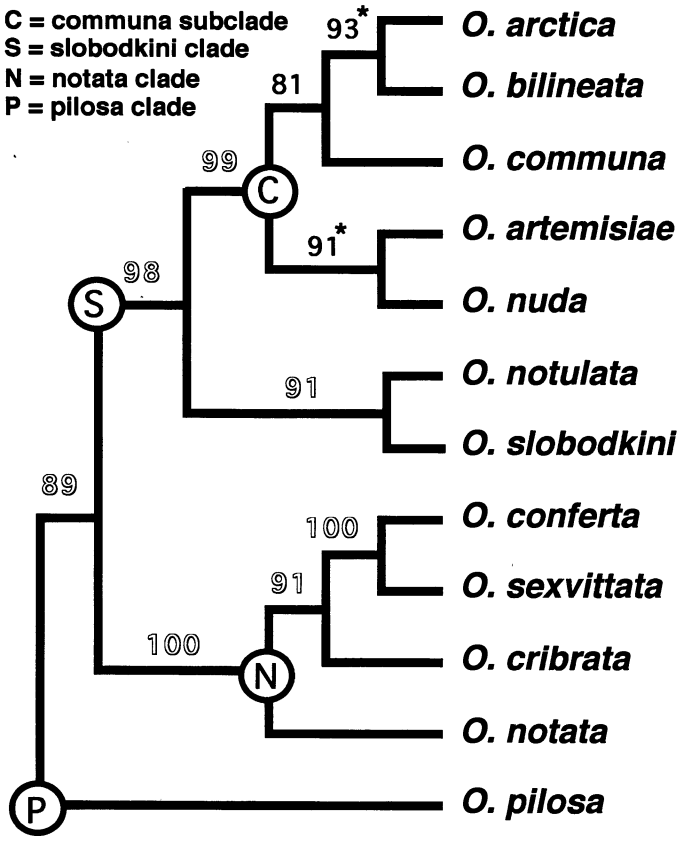

FIG. 1. Phylogenetic relationships among the species of *Ophraella* (for details of analysis, see the text and Funk et al. 1995). Outlined numbers are bootstrap proportions from combined-approach analysis; black numbers are bootstrap proportions from the analysis of mtDNA only, with transversions weighted either 1.1 (asterisks) or 3 times as heavily as transitions. Letters indicate clades referred to in this paper.

MATERIALS AND METHODS

The Study System.—*Ophraella* (Wilcox 1965) is a strictly North American genus of chrysomelid leaf beetles with 14 currently recognized species (Futuyma 1990, 1991; LeSage 1986). All but two (*O. americana* and *O. californiana*) were included in our analysis. *Ophraella* larvae and adults feed on the foliage of composites, with various species recorded from ten genera belonging to four tribes of the Asteraceae (table 1). Individual species range from being strictly monophagous (feeding on a single host plant species) to using particular species from up to five host genera.

Phylogenetic Analysis.—Funk et al. (1995) describe the phylogenetic analysis of 866 base pairs of mitochondrial DNA from the large subunit ribosomal RNA gene (16S, 446 bp) and the cytochrome oxidase subunit I gene (COI, 420 bp), collected from single specimens of each of the 12 *Ophraella* species in the present study. These data were analyzed using generalized parsimony, successive approximations, neighbor-joining, and maximum-likelihood methods, and confidence in the obtained topologies was assessed using the bootstrap (Felsenstein 1985), while a variety of weighting schemes were employed (under parsimony) to test the robustness of the phylogenetic estimate. Additionally, the 64 morphological and 144 allelomorphic characters from F&M were reanalyzed, and all three data sets were ultimately combined in a combined-approach analysis (Kluge 1989). The topology employed in this paper (fig. 1) derives from these analyses. Finally, COI sequence data were collected and analyzed from additional specimens of most species (and from several geographic populations of *O. communa*) to assess intraspecific variation. We interpret some of the resulting phylogeographic patterns in this paper.

Evolutionary Inference.—From our revised phylogenetic estimate, we inferred the history of *Ophraella* host associations by treating the host affiliation of individual *Ophraella*

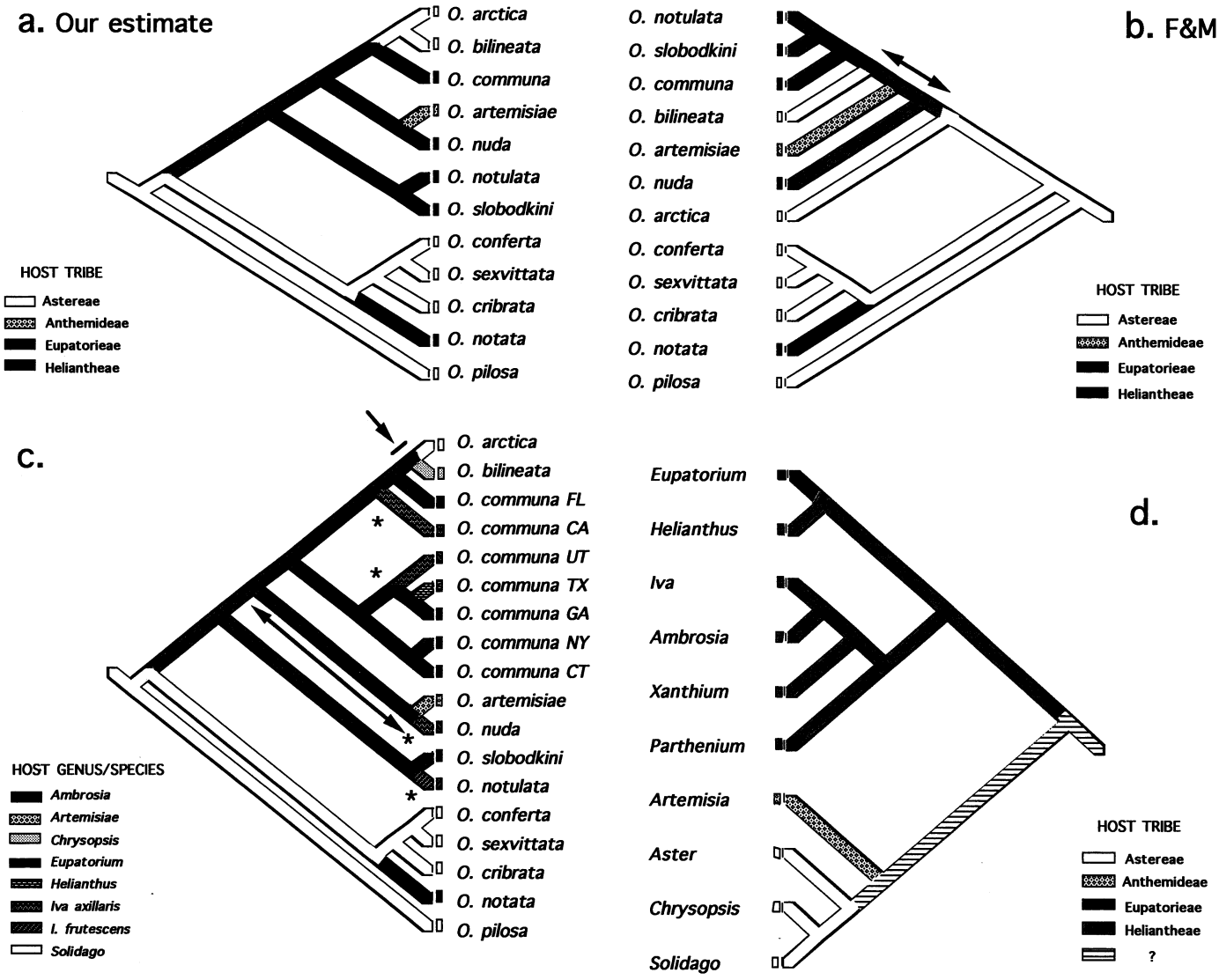

FIG. 2. A history of *Ophraella* host associations. (a) the single unambiguous history of host shifts among host tribes inferred by this study (four steps); (b) one of two equally parsimonious histories inferred by F&M (using the ACCTRAN option in MacClade) (four steps); (c) our estimate of the history of host shifts among host genera and species (using the DELTRAN option in MacClade) (ten steps); (d) phylogenetic relationships among *Ophraella* host plant genera and tribes (see text for citations). Branches for which host optimization is equivocal when all most parsimonious reconstructions are considered are indicated with an arrow. Asterisks in (c) show the four inferred shifts from *Ambrosia* to *Iva*.

species as an unweighted multistate character and obtaining its most parsimonious reconstruction(s) using MacClade 3.01 (Maddison and Maddison 1992). In separate analyses, the tribal identity of host plants was optimized on a tree of *Ophraella* species, and the generic or specific identity of hosts was optimized on a tree which included relationships among various geographic populations of *O. communa*. We compare our inferred history of host use to that of F&M to consider the reinterpretations that they necessitate; we compare it to the phylogenetic relationships of *Ophraella*'s hosts to reexamine the possibility of cospeciation (fig. 2).

B. Farrell (in prep.) provided us with a COI molecular clock of 1.7% sequence divergence per million years, calibrated using palynological and biogeographic data for the genus *Tetraopes* (Cerambycidae). As *Tetraopes* belongs to the sister family of the chrysomelids, clock artifacts attributable to lineage-specific variation in substitution rate (reviewed in, e.g., Martin and Palumbi 1993) are likely to be minimal. This estimate is comparable to the 2.1% estimate inferred for arthropods in a recent survey of mtDNA clocks (Brower 1994 and references therein, e.g., Martin and Simon 1990; DeSalle and Templeton 1992; Knowlton et al. 1993).

Bearing in mind the remaining vagaries associated with molecular clocks (Hillis and Moritz 1990), we estimated divergence times between *Ophraella* clades from the observed range of COI sequence divergences (and their standard errors, provided by MEGA [Molecular Evolutionary Genetic Analysis]; Kumar et al. 1993), between haplotypes from these clades (table 2), corrected for multiple substitutions using Kimura's two-parameter model (Kimura 1980) from sequence

TABLE 2. Percent sequence divergence among COI haplotypes of *Ophraella* species and *Monoxia* sp. (corrected for multiple hits using Kimura's [1980] two-parameter model) (above diagonal) and inferred times of divergence (in millions of years) between all haplotypes (below diagonal). Standard errors are not reported due to space limitations. Abbreviations of specific epithets are those presented in Table 1. Bil.1,2,3 represent three haplotypes of *O. bilineata,* while *O. communa* haplotypes list the state of their origin. See the Materials and Methods for further details.

	arc	bil.1	bil.2	bil.3	com.NY	com.CT	com.GA	com.TX	com.FL	com.UT	com.CA	art.1	art.2	nud	ntl	slb	con	sex	cri	not	pil	Mon
arc		0.7	0.8	1.0	2.2	2.1	1.8	2.8	2.2	3.3	3.5	4.8	5.0	5.3	12.8	16.2	16.7	17.5	15.5	16.2	18.1	16.4
bil.1	0.4		0.5	0.7	2.5	2.3	1.8	2.8	2.5	2.4	3.5	5.0	6.1	6.4	13.2	16.8	17.1	18.5	15.9	16.8	19.9	17.1
bil.2	0.4	0.3		0.8	2.6	2.4	1.8	2.3	2.6	3.4	2.8	5.2	6.2	6.3	13.9	17.8	17.7	18.5	15.8	17.8	19.1	16.7
bil.3	0.6	0.4	0.4		2.7	2.6	2.0	3.0	2.7	3.5	3.3	4.8	6.3	5.9	13.8	17.6	17.5	18.6	16.6	17.2	20.2	17.5
com.NY	1.3	1.5	1.5	1.6		0.3	0.7	1.7	3.0	1.7	2.5	5.0	5.4	5.5	11.8	15.8	16.3	17.0	14.9	17.0	19.5	16.5
com.CT	1.2	1.4	1.4	1.5	0.2		0.5	1.5	3.4	2.0	2.8	4.9	5.4	6.0	12.2	16.1	16.8	17.7	15.2	17.1	20.2	16.5
com.GA	1.0	1.0	1.1	1.2	0.4	0.3		1.0	2.8	1.5	2.8	4.9	5.8	6.5	12.2	15.9	16.5	16.7	15.0	16.8	19.1	16.5
com.TX	1.6	1.6	1.4	1.8	1.0	0.9	0.6		3.2	2.5	2.7	5.8	6.3	6.9	12.8	16.2	17.1	17.6	15.0	17.8	19.1	17.1
com.FL	1.3	1.5	1.5	1.6	1.7	2.0	1.6	1.9		3.8	3.5	6.3	6.8	6.3	13.9	16.4	17.5	18.3	16.0	17.6	19.5	17.8
com.UT	1.9	1.4	2.0	2.1	1.0	1.2	0.9	1.5	2.2		3.7	6.4	7.4	7.4	13.1	16.5	17.4	17.3	15.5	18.0	20.6	18.2
com.CA	2.1	2.1	1.7	1.9	1.4	1.7	1.6	1.6	2.0	2.2		5.8	6.3	5.5	12.4	17.0	17.2	18.0	14.2	16.6	18.5	16.1
art.1	2.8	3.0	3.1	2.8	2.9	2.9	2.9	3.4	3.7	3.7	3.4		4.8	4.2	12.9	16.9	17.5	18.7	15.7	16.3	18.9	16.8
art.2	2.9	3.6	3.6	3.7	3.2	3.2	3.4	3.7	4.0	4.4	3.7	2.8		2.8	14.1	18.6	18.0	18.4	17.0	18.7	20.8	18.6
nud	3.1	3.7	3.7	3.5	3.3	3.5	3.8	4.1	3.7	4.4	3.2	2.5	1.7		14.7	18.8	18.1	19.3	16.9	17.9	21.4	18.9
ntl	7.5	7.8	7.8	8.1	7.0	7.2	7.2	7.5	8.2	7.7	7.3	7.6	8.3	8.7		12.4	14.7	15.0	13.1	16.8	18.1	16.8
slb	9.5	9.9	10.5	10.3	9.3	9.5	9.4	9.6	9.6	9.7	10.0	9.9	11.0	11.0	7.3		14.4	16.4	16.9	16.9	16.7	18.1
con	9.8	10.1	10.4	10.3	9.6	9.9	9.7	10.1	10.3	10.2	10.1	10.3	10.6	10.7	8.6	8.5		2.9	9.6	11.6	16.2	16.9
sex	10.3	10.9	10.9	10.9	10.0	10.4	9.8	10.4	10.8	10.2	10.6	11.0	10.8	11.4	8.8	9.6	1.7		9.9	12.6	16.5	17.5
cri	9.1	9.3	9.3	9.8	8.7	8.9	8.8	8.8	9.4	9.1	8.4	9.2	10.0	9.9	7.7	9.9	5.6	5.8		11.1	17.8	17.4
not	9.5	9.9	10.4	10.1	10.0	10.1	9.9	10.5	10.3	10.6	9.8	9.6	11.0	10.5	9.9	9.9	6.8	7.4	6.6		19.4	20.2
pil	10.7	11.7	11.3	11.9	11.5	11.9	11.3	11.3	11.5	12.1	10.9	11.1	12.2	12.6	10.7	9.8	9.6	9.7	10.4	11.4		17.3
Mon	9.6	10.1	9.8	10.3	9.7	9.7	9.7	10.1	10.4	10.7	9.5	9.9	10.9	11.1	9.9	10.6	9.9	10.3	10.3	11.9	10.2	

data collected in Funk et al. (1995). These divergence estimates allowed an examination of the temporal element of *Ophraella* diversification and host-use evolution.

Results and Discussion

Phylogenetic Analysis

Mitochondrial DNA analysis by all four phylogenetic algorithms yielded the same tree, with generally high bootstrap proportions supporting its nodes (fig. 1). This topology was also quite robust to changes in weighting scheme (for details, see Funk et al. 1995). The separate analyses of the morphology, allozyme, and mtDNA data sets yielded topologies that agreed on all relationships except those within the "slobodkini clade." Combined-approach analysis provided yet stronger bootstrap support for these agreed-upon groupings and confidently placed *O. notulata* and *O. slobodkini* as sister taxa basal to the "communa subclade." However, the combined approach provided inconsistent estimates of relationships within the communa subclade and little bootstrap support for these relationships, possibly because of heterogeneous rates of evolution among data sets (Funk et al. 1995).

The phylogenetic estimate for *Ophraella* that we derived for use in the present study (fig. 1) thus adopts the combined-approach topology for all relationships except those within the communa subclade. For these, the mtDNA topology (see fig. 2c in Funk et al. 1995) was employed as mtDNA provides the only estimates of these relationships that were robust and strongly supported by bootstrap. The *Ophraella* topology that we use thus differs from the mtDNA tree only in the joining of *O. notulata* and *O. slobodkini* as sister taxa. (This grouping was recovered in certain analyses of mtDNA as well, but in others *O. notulata* and *O. slobodkini* were successively basal to the communa subclade.)

History of Ophraella Host Associations

This study was undertaken primarily to provide a more confident estimate of the history of host associations in the genus *Ophraella.* Our reconstruction of this history at the host-tribe level provides an unambiguous estimate, identical in length and in inferred host shifts to the findings of F&M (fig. 2a,b). Under parsimony, the reconstructions from both studies necessitate one host shift from Astereae to Eupatorieae, one from Astereae to Heliantheae, one from Heliantheae to Anthemideae, and one reversal from Heliantheae to Astereae. (F&M had favored this reconstruction over an equally parsimonious alternative on the presumption that one shift from the chemically simple Astereae to the chemically formidable Heliantheae followed by a reversal was more plausible than two independent shifts to Heliantheae.)

New Interpretations.—These similarities, however, belie important reinterpretations of (1) cladistic proximity, (2) lineage-specific host associations, and (3) the timing of realized host shifts that our results require. Examples illustrate each of these: (1) F&M suggested a close relationship between *O. communa* and *O. notulata/O. slobodkini,* whereas we find these taxa to be cladistically and genetically divergent. (2) Although F&M inferred that *O. arctica* has never been associated with Heliantheae hosts, we find that it has only recently (0.8–1.8 mya) shifted to the Astereae after a prolonged Heliantheae affiliation of 4.3–11.7 my. (3) Although both studies agree on the sister species status of *O. notulata* and *O. slobodkini* and on their history of host use, we infer a much more ancient host shift from *Ambrosia* to *Iva* (>5.7 my versus $\ll$ 1 my) by *O. notulata.*

Such findings have important implications for the interpretion of our experimental studies. On the hypothesis that host shifts are constrained by genetic variation, the phylogenetic inference of a particular host shift predicts that the shifted lineage is more likely to exhibit genetic variation in performance on the plant from which it shifted than on plants with which it has never been affiliated (Futuyma et al. 1993, 1995). Genetic variation, however, might be expected to decline over evolutionary time in the absence of selection for its maintenance (Futuyma and Keese 1992; Rausher 1992). Because species that have more recently switched hosts are more likely to retain the genetic variation that facilitated this shift, the capacity to test these predictions may depend on how long ago a shift occurred. Thus, having a confident estimate of realized host shifts and their timing is essential.

Reconstruction at Different Taxonomic Levels.—Our results also illustrate the utility of employing multiple levels of both phylogenetic resolution and character coding in the cladistic study of character evolution. Although tribal host affiliation appears to be relatively conserved, the optimization of host genus and species on a more highly resolved phylogeny reveals a history of frequent host shifts between plants in the Heliantheae (fig. 2c). These include one shift from *Ambrosia artemisiifolia* to *Helianthus* and four independent shifts from *A. artemisiifolia* to *Iva,* three of these to *I. axillaris.* (It is unknown whether *Ambrosia* is also used by the *O. communa* populations for which shifts were inferred.) Given the frequency of these shifts, their specificity, consistent polarity, and occurrence in habitats as diverse as the northern plains (*O. nuda*), the southeast (*O. notulata*) and the arid west (*O. communa*; Utah, California) are striking, and support the notion that constraints do play an important role in determining which shifts are possible. Only by further dissecting the host-plant character and using a more finely resolved phylogeny was the pattern of parallelism exposed. Interestingly, *Ophraella* provides evidence for constraints in terms of the infrequency of shifts at one level of taxonomic resolution and in terms of their frequency at another.

Sources of Ambiguity in Reconstruction.—Though character reconstruction techniques can be used to study a multitude of questions about the origins, history, and correlations of characters (reviewed in Maddison 1994), statements about these inferences must be tempered by an assessment of their reliability. Rigorous methods of assessing this likelihood have not yet been offered. However, robustness of the hypothesis under test to various perturbations of phylogenetic assumptions may provide one measure of confidence (reviewed in Swofford and Maddison 1992; Maddison 1994). One way to do this is by comparing reconstructions on competing topologies. A second is to test the sensitivity of the hypothesis to the addition and deletion of taxa. A third is to compare equally or nearly equally parsimonious reconstructions.

These three approaches suggest that our estimate of tribal

host shifts is robust. As mtDNA provided a single, highly supported tree and a single unambiguous history of shifts, no competing histories are equally parsimonious (although it must be noted that reconstructions entailing but a single extra step provide altered interpretations of character [here, host use] evolution, a distressingly common feature of character optimization). Further, among those (few) alternative relationships found under various weighting schemes (Funk et al. 1995), the only one affecting host-use history placed *O. arctica* and *O. communa/O. bilineata* as sister taxa (a change that yields two equally parsimonious reconstructions, each entailing an additional host shift). Although the addition of a single hypothetical taxon, with each possible host affiliation, to each branch of the topology yielded ambiguous host-use histories in a small proportion of the possible placements, our proposed history was robust to the deletion of any single *Ophraella* species.

We are less confident in the history of shifts among particular host genera and species within tribes by members of the slobodkini clade. COI did not provide enough information (table 2) to support strongly some relationships within *O. communa* (fig. 3). Furthermore, the reconstructed history was not entirely unambiguous (fig. 2c) and was sensitive to the deletion of certain OTUs (operational taxonomic units). Nonetheless, these concerns do not compromise the most important inference drawn from this analysis, namely, that multiple shifts have occurred between *Ambrosia* and *Iva*. Our confidence in the phylogeny of *Ophraella* species (fig. 1) strongly supports the inference of at least three shifts, while the well-supported cladistic separation of the *Iva*-feeding California and Utah populations (fig. 3) of *O. communa* suggests that a fourth shift is likely.

Tempo and Mode of Evolutionary Diversification in Ophraella

Timing of Ophraella Radiation.—Our COI-generated estimates of *Ophraella* divergence events (table 2) are generally considerably more ancient than those inferred by F&M using Nei's calibration (Nei 1987) of allozyme distances. (F&M also pointed out that Sarich (1977) and Thorpe (1982) offered a more extreme calibration; this yields estimates closer to those of COI.) Although F&M found all but the most basal cladogenetic event to have been of Pliocene or Quaternary origin, the COI data imply that all divergences but the communa subclade radiation and the *O. conferta–O. sexvittata* event date to Miocene times, with the *Monoxia–Ophraella* split occurring 8.3–13.4 mya (as compared with the 5.5–7.7 my estimate of F&M). An extreme example of this disparity between estimates is provided by F&M's inference that the slobodkini clade included very closely related species that diverged from one another only 1–2 mya. We estimated the communa subclade itself to be 2.1–4.5 my old, and the divergence between this lineage and that of *O. notulata/O. slobodkini* to have occurred 6.1–12.5 mya. Moreover, the most ancient divergence among *O. communa* haplotypes (2.1 ± 0.6 my) also exceeds the F&M slobodkini clade estimate. The finding that *O. notulata/O. slobodkini* are nearly as genetically divergent from members of the communa subclade (minimally 7.5% overall) as from those of the notata clade (minimally 8.4% overall) suggests that this lineage split off very early in the history of the slobodkini clade. This, in turn, shows that the shift from Astereae to Heliantheae was ancient rather than very recent, and implies a long history of Heliantheae feeding by *Ophraella.*

Discordance between Genetic and Morphological Divergence.—*Ophraella* represents a rather morphologically homogeneous genus of beetles, the species of which differ in fairly subtle aspects. Thus, the discovery of unusually high levels of COI divergence (up to 20.8% among species and 3.8% within *O. communa*) was unexpected, although recent studies of mtDNA evolution in other phytophagous genera report similar findings (Boyce et al. 1994; Brown et al. 1994; B. Farrell in prep.). The decoupling of morphological and molecular evolution in *Ophraella* is further supported by the finding that the morphological sibling species *O. notulata* and *O. slobodkini* (whose species status was first confirmed electrophoretically; Futuyma 1991) appear to have speciated 5.7–8.9 mya. These two species exhibit more than twice as much overall sequence divergence as the most divergent species within the communa subclade. Despite this differentiation, one of the reciprocal crosses between these species produces viable although apparently infertile hybrid offspring in the laboratory (Keese 1994), suggesting that full reproductive isolation has evolved very slowly (cf. Coyne and Orr 1989). Nonetheless, both electrophoretic analysis (F&M) and the monophyly of each species, revealed by our analysis of several populations (Funk et al. 1995), suggest only limited gene flow between them.

An example of the opposite trend is also intriguing. *Ophraella nuda* and *O. artemisiae* are morphologically dissimilar species that exhibited relatively little genetic differentiation in our study. Indeed, *O. artemisiae* appears to be paraphyletic with respect to *O. nuda,* a relationship strongly supported by bootstrap (fig. 3). An even more striking case of the paraphyly of mtDNA lineages is offered by the morphologically coherent *O. communa* with respect to *O. bilineata/O. arctica.* Generalized parsimony and neighbor-joining analyses agree on this paraphyly and neighbor joining supports it with 64 bootstrap replicates. Rather few instances of paraphyletic species (e.g., Avise et al. 1983; Avise et al. 1990; Powell 1991; Melnick et al. 1993; Moran and Kornfield 1993) have yet been reported in the literature.

Mechanisms of Speciation.—Based on the biogeography of morphologically divergent populations and species, Mayr (1954, 1963) suggested that species frequently originate by divergence of localized peripheral populations, a process he later termed peripatric speciation (Mayr 1982). Under this model, new species will often be more closely related to certain populations of the "parent species" than some populations of the latter are to each other. Given the geographic distributions and host affiliations (table 1) of *O. artemisiae* and *O. nuda,* of *O. communa* and *O. bilineata/O. arctica,* and of *O. bilineata* and *O. arctica,* the relationships among their haplotypes in each instance suggest phylogeographic structure (Avise et al. 1987) consistent with the peripatric scenario (fig. 3). In each case, the former taxon has a known distribution that is much larger than, adjacent to, and largely nonoverlapping with, that of the latter taxon, the origin of which is associated with a host shift (fig. 2c). These patterns are

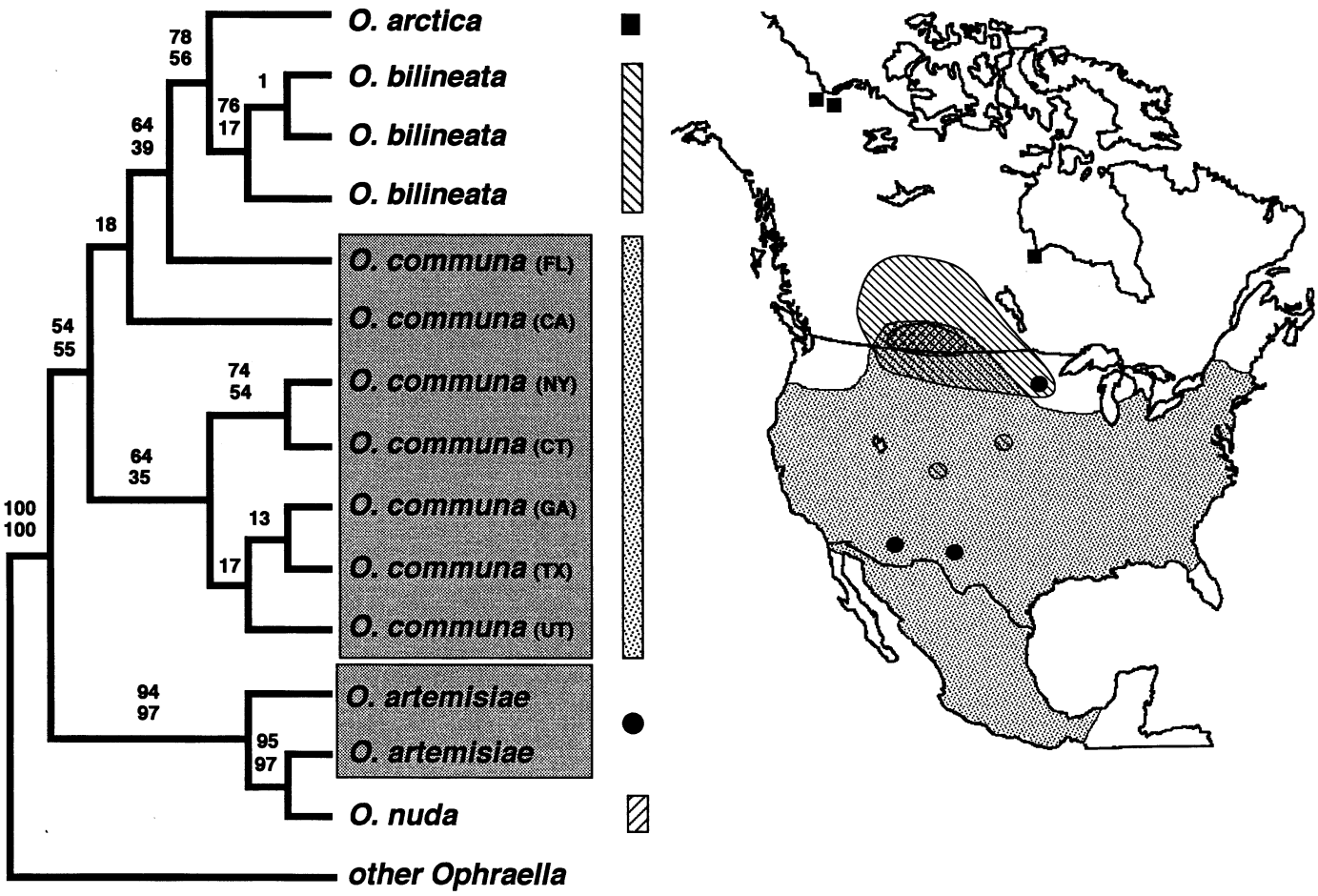

FIG. 3. Cladistic and biogeographic relationships among members of the communa subclade. This tree derives from a 50% majority rule consensus of 100 bootstrap replicates from the unweighted analysis of 420 base pairs of cytochrome oxidase I. Two sets of bootstrap proportions are provided. The lower are from the parsimony analysis used to generate the tree; the upper are from neighbor-joining analysis (where the topologies of the two agree). The topology presented is compatible with the strict consensus of 24 equally shortest trees, the latter differing only in its lack of resolution among the Georgia, Texas, Utah, and New York/Connecticut populations of *Ophraella communa*. The apparent paraphyly of *O. communa* and *O. artemisiae* is highlighted by shading. Patterns following taxon names are those used to illustrate geographic distributions of these species in the accompanying map. *Ophraella arctica* and *O. artemisiae* have been collected from three localities each. Distributions from LeSage (1986), Futuyma (1990), and personal records.

thus consistent with a model of speciation via adaptation of a peripheral isolate to a novel selection regime, yielding rapid differentiation as a by-product.

In a recent study of prodoxid moths, Brown et al. (1994) similarly found evidence suggestive of paraphyletic species in each of two cases in which the derived species was associated with a host shift. Given the current availability of mtDNA sequence data, the phylogeographic approach (Avise et al. 1987), in combination with ecological data such as host associations, provides a potentially powerful means of identifying systems for the study of evolutionary divergence and radiation. In future work, we plan to characterize both the origin of *O. bilineata* from *O. communa* and the nature of the associated host shift.

Other possible explanations for the observed paraphyly certainly cannot be discounted. Hybridization at the geographic borders of these parapatrically distributed species could account for the observed relationships, although the important differences in host association between these forms reduces the plausibility of this argument, as does the apparent lack of gene flow between neighboring populations of *O. notulata* and *O. slobodkini,* which have closely related host plants. Likewise, incomplete lineage sorting (Avise and Ball 1990) of haplotypes from ancestrally polymorphic species remains a viable explanation.

Evidence on Cospeciation.—Within the Asteraceae, *Ambrosia* and *Artemisia* are considered highly derived genera (Heywood et al. 1977; Bremer 1994). Yet, palynological records reveal their existence from at least the Early and Middle Miocene, respectively (Muller 1981). Based on this information, F&M considered *Ophraella* too young to have cospeciated with its hosts. Despite our calculation of a more ancient and largely Miocene history of *Ophraella* diversification, our phylogenetic data provide three classes of evidence which demonstrate that cospeciation does not provide a general explanation for *Ophraella* host associations: (1)

Certain host divergences greatly pre-date beetle divergences, for example, the recent shift from *Ambrosia* to *Artemisia* (1.5–5 my) by *O. artemisiae.* (2) The host loyalty requisite for cospeciation is not universal, for example, the reversal to Astereae and the parallel colonizations of *Iva.* (3) The lack of congruence between host (Jansen et al. 1990, 1991; Kim et al. 1990; Bremer et al. 1992; Bremer 1994) and beetle phylogenies (fig. 2a,d) suggests that even certain host associations with a single origin have been initiated by active host shift rather than by passive cospeciation, for example, the shift of *O. notata* from Astereae to Eupatorieae.

It must also be noted that a number of the asteraceous tribes that include no *Ophraella* hosts are cladistically interspersed with those that do (Bremer 1994), and that only a small fraction of the species within host-including tribes are actually fed upon. Thus, one would have to posit a relictual status for *Ophraella* to argue for an important role for cospeciation even if host-phytophage phylogenies were completely congruent (Brooks and Bandoni 1988). Given that *Ophraella* is a relatively young group with a limited geographic range and a comparably diverse sister genus (*Monoxia,* Blake 1939), this explanation is implausible. Although one might salvage a role for cospeciation by invoking the extinction of lineages with intervening host associations or by positing that previous bouts of parallel cladogenesis have been obscured by more recent host shifts, our study offers little clear evidence for its importance for *Ophraella.*

Our results thus agree with a growing consensus that parallel cladogenesis rarely explains the current host associations of herbivorous insects (Mitter and Farrell 1991; Brown et al. 1994). Although non-phytophagous parasites may indeed commonly cospeciate (Mitter and Brooks 1983), the more facile dispersal of herbivores may dictate that host shifts largely account for macroevolutionary trends of herbivore diversity and host use. We recommend that future studies of host-phytophage systems consider the biogeographic and temporal elements of diversification and host-use evolution as a means of expanding the explanatory scope of the coevolutionary paradigm.

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Appendix

Locality Data

Collection localities and host plants of origin for specimens used in this study. In parentheses are the number of sequenced specimens sharing a COI haplotype for species illustrated in figure 3. Additional specimens were also sequenced for other *Ophraella* species, as reported by Funk et al. (1995).

Ophraella arctica: Canada, N.W.T., Inuvik, *Solidago multiradiata* (1); *O. artemisiae*: Minn., Anoka Co., Bethel, *Artemisia ludoviciana* (2); *O. bilineata*: Canada, Sask., Chaplin, *Chrysopsis villosa* (1,1); Mont., Cascade Co., Cascade, *C. villosa* (1); *O. communa*: Calif., San Diego Co., Kitchen Creek, *Ambrosia psilostachya* (1); Calif., Inyo Co., Antelope Spring, *Iva axillaris* (2, same haplotype as from Kitchen Creek); Conn., Fairfield Co., Reading, *Ambrosia artemisiifolia* (1); Fla., Leon Co., Iamonia, *A. artemisiifolia* (3); Ga., Tift Co., Tifton, *A. artemisiifolia* (1); N.Y., Suffolk Co., Stony Brook (1); Tex., Reeves Co., Balmorhea, *Helianthus ciliaris* (1); Utah, Uintah Co., Vernal, *I. axillaris* (2); *O. conferta*: N.Y., Tompkins Co., Ithaca, *Solidago altissima*; *O. cribrata*: N.Y., Suffolk Co., Manorville, *Solidago juncea*; *O. notata*: N.Y., Tompkins Co., Ithaca, *Eupatorium perfoliatum*; *O. notulata*: S.C., Beaufort Co., Bluffton, *Iva frutescens*; *O. nuda*: Canada, Alta., Pakowki L., *Iva axillaris* (2); *O. pilosa*: N.Y., Tompkins Co., Ithaca, *Aster sagittifolius*; *O. sexvittata*: Fla., Dixie Co., Jena, *Solidago leavenworthii*; *O. slobodkini*: Fla., Leon Co., Iamonia, *Ambrosia artemisiifolia*; *Monoxia* sp.: Fla., Wakulla Co., St. Mark's National Wildlife Refuge, *Lycium carolinense*; *Exema neglecta*: Fla., Tampa, *Baccharis halimifolia.*

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NATURAL SELECTION AGAINST WHITE PETALS IN PHLOX

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Conspicuous variation in corolla color is a striking feature of some populations in a multitude of plant species. Variation in both space and time has been documented in *Justicia simplex* (Jain and Joshi 1962), *Cirsium palustre* (Mogford 1974), *Polygala vulgaris* (Lack and Kay 1987), and *Lotus corniculatus* (Compton et al. 1988). Corolla color polymorphisms most often are generated by mutations at loci regulating pigment synthesis (*Anemone coronaria,* Horovitz and Zohary 1966; *Ipomoea purpurea,* Epperson and Clegg 1987a) and may be spread by gene flow between populations (*Encelia farinosa,* Kyhos 1971; *Epacris impressa,* Stace and Fripp 1977; *Ipomopsis aggregata,* Wilken and Allard 1986; *Phlox drummondii,* Levin and Schmidt 1985), or by mutations at loci regulating pigment synthesis (*Anemone coronaria,* Horovitz and Zohary 1966; *Ipomoea purpurea,* Epperson and Clegg 1987a).

One common type of polymorphism involves rare white-flowered plants in populations of plants with pigmented flowers (e.g., *Delphinium nelsonii,* Waser and Price 1981; *Phlox pilosa,* Levin and Kerster 1970; *Digitalis purpurea,* Ernst 1987; *Echium plantagineum,* Burdon et al. 1983; *Ipomoea purpurea,* Brown and Clegg 1984; *Crocus scepusiensis,* Rafinski 1979). White corollas may be attributed to one recessive gene (*Eschscholzia californica,* Frias et al. 1975; *Lupinus pilosus,* Pazy 1987; *Lupinus nanus,* Horovitz 1969; *Ipomoea purpurea,* Epperson and Clegg 1987a; *Justicia simplex,* Jain and Joshi 1962; *Clarkia xantiana,* Moore and Lewis 1965; *Clarkia unguiculata,* Vasek 1968), to one dominant gene (*Digitalis purpurea,* Ernst 1987; *Raphanus raphanistrum,* Stanton et al. 1989), or to multiple genes (Lawrence and Price 1940; Grant 1975). The genetic basis for white petals may vary among plants within the same population (*Endymion non-scriptus,* Stickland and Harrison 1977).

Corolla color variation is of particular interest, because pollinators have keen color vision (Kevan 1983) and can differentiate between corolla color variants (Kay 1978). Their ability to differentiate may result in assortative pollination (Kay 1976, 1982; Levin and Watkins 1984) or discrimination against certain variants (Levin 1972a, Waser and Price 1983). Variants discriminated against will be at a selective disadvantage as a result of lower seed-set (Harding 1970; Waser and Price 1981) or lower paternity (Stanton et al. 1989). These variants also may have higher selfing rates and thus be more inbred than favored variants (Brown and Clegg 1984).

It is tempting to assume that the scarcity of white-flowered plants in local populations is due to pollinator-mediated selection against them. One striking example of such selection is in *Delphinium nelsonii,* where white-flowered plants produce substantially fewer seeds per flower than plants with pigmented flowers (Waser and Price 1981). Pollinators undervisit albino flowers, because their nectar rewards are more time-consuming to obtain than those of pigmented flowers (Waser and Price 1983).

The present study explores the basis for the paucity of white-flowered plants in *Phlox drummondii* Hook. Most populations contain only plants with pigmented petals. A small percentage of populations contain white-flowered plants, which rarely constitute over 1% of the population. This *Phlox* is pollinated by butterflies, moths, and hawkmoths, which can differentiate between white and other colors (Levin and Schaal 1970; Levin 1972a), and in garden trials may discriminate against white-flowered plants (Levin 1972a).