Phylogeny of Greya (Lepidoptera: Prodoxidae), Based on Nucleotide Sequence Variation in Mitochondrial Cytochrome Oxidase I and II: Congruence with Morphological Data

Jonathan M. Brown, * Olle Pellmyr, † John N. Thompson, ‡ and Richard G. Harrison§
*Department of Biology, Bucknell University; †Department of Biological Sciences, University of Cincinnati; ‡Departments of Botany and Zoology, Washington State University; and §Section of Ecology and Systematics, Cornell University

The phylogeny of Greya Busck (Lepidoptera: Prodoxidae) was inferred from nucleotide sequence variation across a 765-bp region in the cytochrome oxidase I and II genes of the mitochondrial genome. Most parsimonious relationships of 25 haplotypes from 16 Greya species and two outgroup genera (Tetragma and Prodoxus) showed substantial congruence with the species relationships indicated by morphological variation. Differences between mitochondrial and morphological trees were found primarily in the positions of two species, G. variabilis and G. pectinijku, and in the branching order of the three major species groups in the genus. Conflicts between the data sets were examined by comparing levels of homoplasy in characters supporting alternative hypotheses. The phylogeny of Greya species suggests that host-plant association at the family level and larval feeding mode are conservative characters. Transition/transversion ratios estimated by reconstruction of nucleotide substitutions on the phylogeny had a range of 2.0–9.3, when different subsets of the phylogeny were used. The decline of this ratio with the increase in maximum sequence divergence among taxa indicates that transitions are masked by transversions along deeper internodes or long branches of the phylogeny. Among transitions, substitutions of A→G and T→C outnumbered their reciprocal substitutions by 2–6 times, presumably because of the approximately 4:1 (77%) A+T-bias in nucleotide base composition. Of all transversions, 73%–80% were A→T substitutions, 85% of which occurred at third positions of codons; these estimates did not decrease with an increase in maximum sequence divergence of taxa included in the analysis. The high frequency of A→T substitutions is either a reflection or an explanation of the 92% A+T bias at third codon positions.

Introduction

Mutualistic interactions between plants and their pollinating seed parasites are often cited as examples of strongly coevolved interactions. Together with figs and fig wasps (Ramirez 1970; Janzen 1979; Wiebes 1979; Kjellberg et al. 1987; Bronstein 1989; Herre 1989), yuccas (Yucca: Agavaceae) and the true yucca moths (Tegeticula: Prodoxidae) comprise perhaps the classic cases of coevolved mutualisms thought to have evolved from antagonistic interactions (Riley 1892; Powell and Mackie 1966; Davis 1967; Aker and Udovic 1981; Thompson 1982; Powell 1983; Addicott 1986). Although ecological studies of these systems abound, the origins of behavioral, morphological, and life-history characters crucial to the development of pollination mutualisms have not been examined in a phylogenetic framework.

Recent ecological studies of the genus Greya, the putative sister taxon to the Agavaceae-feeding prodoxids (Wagner and Powell 1988), have identified species that pollinate their host plants, yet, like the yucca moths, are seed parasites that destroy a small proportion of the host's developing seeds (Davis et al. 1992; Pellmyr and Thompson 1992; Thompson and Pellmyr 1992). Greya are univoltine insects associated with plants in the families Saxifragaceae and Umbelliferae; species with known hosts are strict local host specialists that feed on a single host-plant species at any one site but show variation in host association among populations (table 1; Davis et al. 1992). Davis et al. (1992) revised the genus Greya, adding eight new species, and proposed a tentative phylogeny based on cladistic analysis of 28 morphological characters (fig. 1). Two species groups were strongly supported by multiple synapomorphies: (1) the punctiferella group, consisting of G. punctiferella, G. piperrerla, G. mitella, and G. obscura, which includes all species known to feed on meristematic tissue, and (2) the solenobiella group, consisting of G. solenobiella, G. suffusca, G. reticulata, G. powelli, and G. subalba, which...
includes all species known to feed on plant species in the family Umbelliferae (table 1). In contrast to the clear support for these two groups, other species groups were not strongly supported. Furthermore, the branching order of the species groups can be altered with only minor changes in overall branch lengths. A robust phylogeny of Greya species that is based on a greater number of characters that are independent of the insect-plant interactions is needed as a framework for understanding the sequence of changes in behavioral, morphological, and life-history characters which have led, as in their yucca moth relatives, to strong and specialized interactions between some Greya species and their hosts.

Pellmyr and Thompson (1992) have suggested at least two separate origins of mutualism in the Prodoxidae. Two traits, local host specialization and mating on the host, are found in all prodoxid genera for which information is available. Oviposition into the flower is found in species of at least four genera, and, in three of these genera, some species also pollinate their host. Unfortunately, because of a paucity of morphological synapomorphies, traditional phylogenies of the Prodoxidae rely on such life-history and host-association characters.

**Table 1**

Hosts, Ranges, and Site Locations for *Greya* mtDNA Haplotypes

<table>
<thead>
<tr>
<th>Moth Species</th>
<th>Host Species</th>
<th>Range</th>
<th>mtDNA Site(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. punctiferella</em></td>
<td><em>Tiarella trifoliata</em> (S)</td>
<td>Southeastern Ala. to northern Calif.</td>
<td>Clallum County, Wash.</td>
</tr>
<tr>
<td></td>
<td><em>Tolmiea menziesii</em> (S)</td>
<td>Southern British Columbia to central Calif.</td>
<td>Glacier County, Mont.</td>
</tr>
<tr>
<td></td>
<td><em>Tellima grandiflora</em> (S)</td>
<td>Coastal range from southwestern Oreg. to southern Calif.</td>
<td>Mendocino County, Calif. and Santa Barbara County, Calif.</td>
</tr>
<tr>
<td><em>G. piperella</em></td>
<td><em>Heuchera cylindrica</em> (S)</td>
<td>Northwestern Idaho and southeastern Wash.</td>
<td>Latah County, Idaho</td>
</tr>
<tr>
<td></td>
<td><em>H. micrantha</em></td>
<td>West coast of North America from British Columbia to southern Calif.</td>
<td>Tulare County, Calif. and Whitman County, Wash.</td>
</tr>
<tr>
<td><em>G. mitellae</em></td>
<td><em>Mitella stauropetala</em> (S)</td>
<td>Unknown</td>
<td>Benewah County, Idaho</td>
</tr>
<tr>
<td><em>G. obscura</em></td>
<td><em>Lithophragma affine</em> (S)</td>
<td>Unknown</td>
<td>Umatilla County, Oreg.</td>
</tr>
<tr>
<td></td>
<td><em>L. cymbalaria</em></td>
<td>Known</td>
<td>Glacier County, Mont. and Whitman County, Wash.</td>
</tr>
<tr>
<td></td>
<td><em>L. heterophyllum</em></td>
<td>Known</td>
<td>Glacier County, Mont. and Whitman County, Wash.</td>
</tr>
<tr>
<td></td>
<td><em>L. parviflorum</em></td>
<td>Known</td>
<td>Glacier County, Mont. and Whitman County, Wash.</td>
</tr>
<tr>
<td></td>
<td><em>L. tenellum</em></td>
<td>Known</td>
<td>Glacier County, Mont. and Whitman County, Wash.</td>
</tr>
<tr>
<td></td>
<td><em>H. grossulariifolia</em></td>
<td>Known</td>
<td>Glacier County, Mont. and Whitman County, Wash.</td>
</tr>
<tr>
<td><em>G. enchrysa</em></td>
<td><em>H. cylindrica</em> (S)</td>
<td>Rocky Mountains from southern British Columbia to central Oreg.</td>
<td>Glacier County, Mont. and Umatilla County, Oreg.</td>
</tr>
<tr>
<td></td>
<td><em>H. grossulariifolia</em></td>
<td>Known</td>
<td>Clallum County, Wash.</td>
</tr>
<tr>
<td><em>G. variabilis</em></td>
<td>Unknown</td>
<td>Ala. to coastal Wash.</td>
<td>Clallum County, Wash.</td>
</tr>
<tr>
<td><em>G. pectinifera</em></td>
<td>Unknown</td>
<td>Clallum County, Wash.</td>
<td>Clallum County, Wash.</td>
</tr>
<tr>
<td><em>G. variata</em></td>
<td>Unknown</td>
<td>Glacier County, Mont. and southern Alberta</td>
<td>Clallum County, Wash.</td>
</tr>
<tr>
<td><em>G. subalba</em></td>
<td><em>Lomatium ambiguum</em> (U)</td>
<td>Southern Oreg. to southern British Columbia</td>
<td>Glacier County, Mont. and Whitman County, Wash.</td>
</tr>
<tr>
<td></td>
<td><em>L. dissectum</em></td>
<td>Known</td>
<td>Clallum County, Wash.</td>
</tr>
<tr>
<td></td>
<td><em>L. grayi</em></td>
<td>Known</td>
<td>Clallum County, Wash.</td>
</tr>
<tr>
<td></td>
<td><em>L. triternatum</em></td>
<td>Known</td>
<td>Clallum County, Wash.</td>
</tr>
<tr>
<td></td>
<td><em>L. macrocarpum</em></td>
<td>Known</td>
<td>Clallum County, Wash.</td>
</tr>
<tr>
<td><em>G. solenobiella</em></td>
<td><em>Yabea microcarpa</em> (U)</td>
<td>Southwestern Oreg. to southern Calif.</td>
<td>Tulare County, Calif.; Santa Clara County, Calif.; and Butte County, Calif.</td>
</tr>
<tr>
<td><em>G. suffusa</em></td>
<td><em>Osmorhiza brachypoda</em> (U)</td>
<td>Tulare County, Calif.</td>
<td>Tulare County, Calif.</td>
</tr>
<tr>
<td><em>G. reticulata</em></td>
<td><em>O. chilensis</em> (U)</td>
<td>Southern Calif.</td>
<td>Santa Clara County, Calif.</td>
</tr>
<tr>
<td><em>G. powelli</em></td>
<td><em>Bowlesia incana</em> (U)</td>
<td>Southern Calif.</td>
<td>Tulare County, Calif.</td>
</tr>
</tbody>
</table>

*S* and *U* refer to members of the Saxifragaceae and Umbelliferae plant families, respectively. Hosts are those for which ovipositions have been observed and confirmed through dissections of plant tissues by O.P. and/or J.N.T.
Fig. 1.—Phylogeny of *Greya* Busck, based on 28 morphological characters. Unambiguous character changes are shown on one of five MP trees. Equally parsimonious positions of *G. variata* are as sister to the *solenobiella group* and as sister to the *solenobiella group* + (*G. politella* + *G. enchrysa* + *G. variabilis* + *G. punctiferella*). Letters a-e indicate character states 0–5 for multistate characters. Reversals are indicated by a hyphen.

for inferring relationships (Wagner and Powell 1988); this makes the phylogenetic study of these same characters somewhat circular. We describe here a reexamination of the phylogenetic relationships within *Greya* (the putative sister genus to the Agavaceae-feeding genera), using variation in mitochondrial DNA (mtDNA) sequences, which provide characters presumably independent of life-history, morphological, and behavioral evolution.

Despite the proliferation of phylogenetic studies based on nucleic acid sequence variation, many issues regarding the most accurate methods for reconstructing phylogenies with such data remain in dispute (for a review of methods, see Swofford and Olsen 1990). For example, many studies of mtDNA divergence indicate differential rates of evolutionary change at different nucleotide positions (e.g., synonymous vs. nonsynonymous changes) and for different substitutions (e.g., transitions vs. transversions) (Brown et al. 1982; Wolstenholme and Clary 1985; DeSalle et al. 1987). Different rates of evolution among characters may result in greater homoplasy in rapidly evolving character sets, leading some researchers to suggest some form of a priori or a posteriori character and/or substitution weighting (Felsenstein 1981; Williams and Fitch 1989; Swofford and Olsen 1990; Wheeler 1990). Unfortunately, there is no consensus about the most appropriate methods for character weighting. Using a data set that spans a range of pairwise sequence divergences from 0.1%–17%, we compare results of different methods of character (position) and substitution weighting on the phylogeny of *Greya*.

**Material and Methods**

Specimens were collected during 1988–91 by O.P. and J.N.T. and were shipped live to Ithaca, N.Y. for storage at −80°C. Seven *Greya* species with widespread geographic range are represented by individuals from more than one population; nine species with limited geographical distributions are represented by single individuals (for site locations, see table 1). One species, *G. sparsipunctella* (Walsingham), was excluded from both the morphological and molecular analyses, as it has not been collected since its discovery in 1871 and because males are unknown. As outgroups, we included...
haplotypes from two other prodoxid species, *Tetragma gei* and *Prodoxus quinquepunctellus*.

We extracted total DNA from specimens by using a modification of the protocols of Harrison et al. (1987). Using the polymerase chain reaction (PCR) (Saiki et al. 1988) and total DNA as template, we amplified a region of the mtDNA genome, including most of the cytochrome oxidase I and II (COI and COII, respectively) genes and the intervening leucine tRNA. We used a Perkin Elmer Cetus thermal cycler and the following cycle profiles: 30 s at 93°C, 60 s at 47°C, and 90 s at 72°C, for 30 cycles. We performed a second, asymmetric PCR (Gyllensten and Ehrlich 1988) with a single primer to produce single-stranded DNA for sequencing. We sequenced by dideoxy chain termination (Sanger et al. 1977), using 35S and the Sequenase version 2.0 DNA sequencing kit (U.S. Biochemical).

We used nine primers to generate double- and single-stranded DNA: S1751, 5’GGATCACCTGATATAGCATTCCC; S1859, 5’GGAAACGGATGAAAC(A/T)-GTNTA(C/T)CGCC; S2183, 5’CAACATTATTTTGATTITTTTG; S2792, 5’ATACCTGACGGTATTCAGAA; S2840, 5’TATTTCCTTTTCTTAGG(A/G)TC; A3389, 5’TCATAAGTCTT(A/G)ATATCATTG; A3568, 5’CCTAAGGA(A/T)GGAAT(A/T)GTCTTCA; A3661, 5’CCACAAATTTCTGTAACATTGACCA; and A3772, 5’GAGACCCCTGCTTTGAGTCATCT; “S” and “A” refer to sense and antisense strands, and numbers refer to the position of the 3’ end (numbering is based on *Drosophila yakuba* sequence; Clary and Wolstenholme 1985). Primers were designed by members of the Richard Harrison lab at Cornell University on the basis of comparisons of published sequence from *D. yakuba* (Clary and Wolstenholme 1985) and *Apis mellifera* (Crozier et al. 1989) as entered in GenBank (Genetics Computer Group 1991). Primers used for sequencing were S2792, A3389, A3602, and A3661, which resulted in a continuous sequence of 765 bp spanning nucleotide position 2852 in COI to position 3622 in COII (numbers as above).

We used Genetics Computer Group’s (1991) GCG programs to enter and align sequences from all taxa. We used PHYLIP 3.4 DNADISTANCE program (Felsenstein 1991) to generate pairwise corrected haplotype distances, using Kimura’s (1980) two-parameter model (transitions given twice the probability of transversions). We used PAUP 3.1.1 (Swofford 1993) to search for most parsimonious (MP) trees of haplotype (for mtDNA) and species (for morphology) relationships. All searches of the mtDNA data matrix were performed with the HEURISTIC option, since the large number of taxa precluded the use of exact algorithms for finding MP trees. However, random addition with 10 replicates was used to generate initial trees for the TBR branch-swapping routine. This method increases the probability of finding the MP trees with these heuristic algorithms (Swofford 1991). Searches using the morphological character matrix were made with the BRANCH AND BOUND option of PAUP. Length penalties (i.e., the difference in total tree length) for alternative topologies not returned in searches for MP trees were obtained by defining “constraint trees” and enforcing these constraints during a heuristic search in PAUP (Swofford 1991); these penalties were verified using MacClade 3.0 (Maddison and Maddison 1992).

We also analyzed the mtDNA matrix by using different forms of character and substitution weighting: (1) For character weighting, PAUP’s REWEIGHT option was used to implement Farris’s successive weighting method, which reweights characters in proportion to their rescaled consistency index on an initial tree (we used the MP evenly weighted trees); this procedure is repeated until a single topology is consistently supported (Farris 1988). Because of the extreme differences in variability at different positions within codons (table 2), we also chose relative weights of 1:2, 1:5, and 0:1 for third-position changes versus changes at all other positions (including in tRNA^-Leu^). (2) For substitution weighting, we performed searches requiring different length “penalties” for different nucleotide substitutions by defining step matrices for each model of substitution weights (Swofford 1991). First, we chose relative weights of transversions versus transitions of 2:1–10:1. We also used 1:0 (transversions:transitions) weighting to search

### Table 2

**Percent Variable Sites, by Region and Codon Position, across 27 *Greya* and Two Outgroup Genera Haplotypes**

<table>
<thead>
<tr>
<th></th>
<th>FIRST</th>
<th></th>
<th></th>
<th>SECOND</th>
<th></th>
<th></th>
<th>THIRD</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COI</td>
<td>COII</td>
<td></td>
<td>COI</td>
<td>COII</td>
<td></td>
<td>COI</td>
<td>COII</td>
</tr>
<tr>
<td>Constant (%)</td>
<td>63</td>
<td>71</td>
<td></td>
<td>81</td>
<td>91</td>
<td></td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>Uninformative (%)</td>
<td>12</td>
<td>12</td>
<td></td>
<td>9</td>
<td>5</td>
<td></td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Informative (%)</td>
<td>25</td>
<td>18</td>
<td></td>
<td>5</td>
<td>4</td>
<td></td>
<td>56</td>
<td>17</td>
</tr>
<tr>
<td>No. of sites</td>
<td>52</td>
<td>180</td>
<td></td>
<td>53</td>
<td>180</td>
<td></td>
<td>53</td>
<td>180</td>
</tr>
</tbody>
</table>

We used PHYLIP 3.4 DNADISTANCE program (Felsenstein 1991) to generate pairwise corrected haplotype distances, using Kimura’s (1980) two-parameter model (transitions given twice the probability of transversions). We used PAUP 3.1.1 (Swofford 1993) to search for most parsimonious (MP) trees of haplotype (for mtDNA) and species (for morphology) relationships. All searches of the mtDNA data matrix were performed with the HEURISTIC option, since the large number of taxa precluded the use of exact algorithms for finding MP trees. However, random addition with 10 replicates was used to generate initial trees for the TBR branch-swapping routine. This method increases the probability of finding the MP trees with these heuristic algorithms (Swofford 1991). Searches using the morphological character matrix were made with the BRANCH AND BOUND option of PAUP. Length penalties (i.e., the difference in total tree length) for alternative topologies not returned in searches for MP trees were obtained by defining “constraint trees” and enforcing these constraints during a heuristic search in PAUP (Swofford 1991); these penalties were verified using MacClade 3.0 (Maddison and Maddison 1992).

We also analyzed the mtDNA matrix by using different forms of character and substitution weighting: (1) For character weighting, PAUP’s REWEIGHT option was used to implement Farris’s successive weighting method, which reweights characters in proportion to their rescaled consistency index on an initial tree (we used the MP evenly weighted trees); this procedure is repeated until a single topology is consistently supported (Farris 1988). Because of the extreme differences in variability at different positions within codons (table 2), we also chose relative weights of 1:2, 1:5, and 0:1 for third-position changes versus changes at all other positions (including in tRNA^-Leu^). (2) For substitution weighting, we performed searches requiring different length “penalties” for different nucleotide substitutions by defining step matrices for each model of substitution weights (Swofford 1991). First, we chose relative weights of transversions versus transitions of 2:1–10:1. We also used 1:0 (transversions:transitions) weighting to search
for trees with "transversion parsimony," which ignores transitional changes in calculation of tree lengths (Swoford and Olsen 1990). We performed searches that ignored all silent mutations by translating the protein-coding sequences to amino acids, using the TRANSLATE option of GCG and the Drosophila mtDNA code. Finally, we used MacClade 3.0 (Maddison and Maddison 1992) to infer the numbers of different substitutions on the MP evenly weighted trees (ambiguous changes are averaged). We compared estimates calculated using different subgroups of Greya species which varied in maximum pairwise sequence divergence, in order to explore the effects of multiple substitutions. We used MacClade's CHART TO TYPE option to translate substitution frequencies into phylogenetic weights (Williams and Fitch 1989, 1990; Wheeler 1990). We also reanalyzed the morphological data, including recently acquired data reported as missing by Davis et al. (1992) (see Appendix).

Results

Morphological Phylogeny

When the morphological character-state matrix was used (see Appendix), a BRANCH AND BOUND search in PAUP, returned five MP trees of 49 steps (fig. 1). As in a different analysis (Davis et al. 1992), two major species groups, the punctiferella and solenobiella groups, are strongly supported by multiple synapomorphies. A third species group, including Greya politella, G. pectinifera, G. variabilis, and G. enchrysa, is supported by two synapomorphies, but species in these groups exhibit many reversals. The MP trees differed in the placement of G. variata and in relationships within the solenobiella species group. The positions of the punctiferella and politella groups can be switched with only a single step added in tree length.

mtDNA Variation

Sequences from 27 mtDNA haplotypes were aligned by eye, with no evidence of indels. Over the entire 765-bp region, 63% of the nucleotide positions were constant, 10% were uninformative (i.e., any variants were found in single haplotypes), and 27% were informative. Third positions in codons were most variable, whereas second positions were least variable (table 2). A's or T's were found at 77% of sites across all haplotypes, with A+T bias particularly pronounced (92%) at third positions in codons (table 3). Estimated sequence divergences (corrected for multiple substitutions) between some pairs of Greya species were as large as 16% (aligned sequences and distance matrix are available from the corresponding author on request). Greya obscuromaculata, G. pectinifera, G. variata, and G. variabilis were each distinct from all other Greya species, with these interspecific distances often only slightly less than those between Greya species and outgroup genera. Estimated sequence divergence between haplotypes from different populations of widespread species was from less than 1% to as much as 5.7% (for two G. obscura haplotypes from Santa Barbara and Mendocino Counties in California). In contrast, maximum estimated divergence among all haplotypes of three related species, G. punctiferella, G. piperella, and G. mitellae, was less than 3%.

A heuristic search using equal weighting of all nucleotide substitutions returned two MP trees of 718 steps that differed only in the position of G. powelli (fig. 2). The trees support the monophyly of three distinct species groups. However, relationships among the three major species groups could be altered with only minimal change in tree length; for example, switching the positions of the punctiferella group (G. obscura + G. punctiferella + G. mitellae + G. piperella) and the politella group (G. politella + G. enchrysa) added only a single step to the length of the tree, while switching the positions of the politella group and G. obscuromaculata + G. solenobiella group (G. solenobiella + G. suffusca + G. powelli + G. reticulata + G. subalba) required three extra steps. The position of G. obscuromaculata could be switched from its position as the basal member of the solenobiella group to a basal position in the punctiferella group (as is suggested by the morphological phylogeny) by the addition of a single step in tree length.

Estimates of transition/transversion ratios based on reconstructed changes over one of the MP trees were from 2.0 (calculated over all haplotypes; table 4) to 9.3 (calculated in the punctiferella group only) and increased as the maximum pairwise sequence divergence of haplotypes included in the analysis decreased (fig. 3). A→G and T→C substitutions outnumbered their reciprocal substitutions by 4–5 times (table 4; this range was 2:1–6:1, depending on the group of species over which substitutions were reconstructed). T→C transitions out-

Table 3

<table>
<thead>
<tr>
<th>CODON POSITION</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>tRNA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.385</td>
<td>0.278</td>
<td>0.454</td>
<td>0.353</td>
<td>0.370</td>
</tr>
<tr>
<td>G</td>
<td>0.157</td>
<td>0.097</td>
<td>0.020</td>
<td>0.135</td>
<td>0.096</td>
</tr>
<tr>
<td>C</td>
<td>0.167</td>
<td>0.185</td>
<td>0.063</td>
<td>0.132</td>
<td>0.138</td>
</tr>
<tr>
<td>T</td>
<td>0.291</td>
<td>0.440</td>
<td>0.463</td>
<td>0.380</td>
<td>0.396</td>
</tr>
</tbody>
</table>
FIG. 2. One of two most parsimonious trees of mtDNA haplotype relationships, based on equal weighting of all nucleotide substitutions (the alternative MP tree places Greya powelli as sister to G. solenobiella + G. suffusca). Branch lengths are proportional to the number of unambiguous changes. Bootstrap percentages for nodes >50% are indicated.

numbered A→G transitions by 2:1–3:1. A→T substitutions accounted for 80% of all transversions; this ratio decreased slightly as the maximum sequence divergence of haplotypes included decreased. Eighty-five percent of these A→T substitutions occurred at third codon positions.

Character Weighting

Giving changes at third codon positions a weight of one, while giving all other changes weights of up to five, resulted in the same two parsimonious trees found in the evenly weighted analysis. Throwing out information at third positions (i.e., giving them zero weight) resulted in a large number of MP trees, with little or no resolution of branches among closely related species.

Farris's (1988) method of successive weighting converged after a single round on the MP (evenly weighted) tree that places G. powelli as basal to the remaining members of the solenobiella group (fig. 2). In summary, heavier weighting of characters (nucleotide positions) suspected to be more phylogenetically conservative supported the topology obtained when characters were evenly weighted.

Substitution Weighting

Since transversions appear to accumulate more slowly than transitions (fig. 3), heavier weighting of the former may indicate more clearly the relationships of highly divergent taxa. Giving transversions twice the
weight of transitions (7:1 transversions:transitions) returned the two MP trees of the evenly weighted analysis (fig. 2). Searches using weights greater than 5:1 returned two MP trees that differed from the evenly weighted MP trees only in the relative positions of the punctiferella and politella (G. politella + G. enchrysa) species groups and in the placement of G. pectinifera and G. variabilis as sister species. When the DNA sequences were translated, 64 (28%) of 232 amino acids were variable across the 27 haplotypes. Since only 36 of these variable positions were informative, a heuristic search returned nine MP trees that agreed only on a few nodes within Greya.

Weight matrices calculated by MacClade 3.0 (Maddison and Maddison 1992) on the basis of reconstructed substitution frequencies after the methods of Wheeler (1990) and Williams and Fitch (1990) consistently violated the triangle inequality; for example, when phylogenetic weights are calculated as the reciprocal of substitution frequencies, a substitution G to C (cost of 10 steps) is more costly than the sum of three intermediate substitutions (G-->A [cost of 2.1] + A-->T [cost of 1.1] + T-->C [cost of 1.0] = 4.2 steps; table 5). For this reason, we chose not to use them as input matrices in PAUP searches.

Congruence of Morphological and Mitochondrial Hypotheses

The two data sets provided largely corroborating hypotheses about species relationships and differed primarily in the positions of G. pectinifera and G. variabilis (fig. 4). Fitting the morphological characters on the mitochondrial MP trees required 10 extra steps, an increase of 20%; fitting the molecular characters on the morphological MP trees required 26–35 extra steps, an increase of 3.6%-4.8%. Two measures of incongruence between the two sets of characters were calculated: $I_{MP} = 0.026$ (Mickevich and Farris 1981), and $I_M = 0.074$ (Kluge 1989).

Total Evidence

A heuristic search of a data set combining both sets of characters resulted in a single MP tree that differed

![Transition bias](image)

**FIG. 3.—**Transition bias (transitions/transversions) and the proportion of transversions which are A+T substitutions reconstructed over trees of different subsets of taxa. Twelve subsets were created, by sequentially pruning distant taxa from the previous group, as follows: all taxa; Tetragona; Prodoxus; G. pectinifera; G. obscuromaculata; G. variata; G. variabilis; G. politella, and G. enchrysa; solenobiella group only; G. reticulata; G. subalba; punctiferella group only.

![Comparison of morphological and mitochondrial MP trees](image)

**FIG. 4.—**Comparison of morphological and mitochondrial MP trees.
from the molecular MP trees only in the position of *G. obscuromaculata*, which was placed, as in the morphological MP trees, as sister to the *punctiferella* group.

**Discussion**

Phylogenetic studies of molecular genetic variation are increasingly being used to test evolutionary hypotheses about morphological, behavioral, and life-history evolution. In particular, the reconstruction of the evolutionary history of ecological interactions is most robust when phylogenies are based on characters independent of phenotypic characters involved in, or affected by, the history of these interactions (Coddington 1988; Brooks and McLennan 1991; Armbruster 1992; but see Maddison and Maddison 1992). Although suitable morphological characters may be found that resolve relationships and provide an independent template for testing evolutionary hypotheses about other phenotypic characters, mitochondrial genes provide a wealth of variation almost certainly independent of changes in ecologically relevant characters. This variation may be particularly useful in generating phylogenies in groups of taxa in which most variation in morphological characters is associated with apparent adaptation (such as in groups that have undergone recent adaptive radiation), or where excessive homoplasy in such morphological characters is apparent.

*Greya* species provide an example of taxa in which molecular genetic variation can inform morphologically based studies (and vice versa) by identifying characters that are unlikely to indicate phylogenetic relationships. Homoplasy in many morphological characters used by Davis et al. (1992) to reconstruct the relationships within the genus *Greya* resulted in uncertainty of the placement of certain species (e.g., *G. variata*, fig. 1). Several morphological traits that define monophyletic groups within *Greya* are also found in other prodoxid genera. For example, character 2 (see Appendix), the number of segments of the maxillary palpus, has evolved from the plesiomorphic state (five segments) to the apomorphic state (three to four segments) independently at least twice among the prodoxids, as it is found in the *solenobiella* group of *Greya* and in species of the prodoxid genera *Tetragma*, *Prodoxoides*, *Tridentaforma*, and *Parategeticula* (Davis et al. 1992). Similarly, the zigzag wing pattern (character 8) that also defines the *solenobiella* group has evolved at least twice more in the family (in species of the genera *Lampronida* and *Prodoxus*). The mitochondrial phylogeny may be used as an independent test of morphological characters that conflict in their indications of phylogenetic affinity. Differences between rates of evolution in morphological and molecular characters may also allow morphological evidence to provide substantial support for nodes that are supported by few molecular synapomorphies, e.g., nodes defining the branching order of lineages that diverged over a short period of time. We first compared conflicts between the hypotheses of the two separate data sets by examining the evidence for conflicting nodes.

**Basal Taxa**

The relatively basal positions of three taxa, *G. variata*, *G. variabilis*, and *G. pectinifera*, were strongly indicated by the mtDNA phylogeny; constraining the latter two species to their positions in the morphological MP tree required 20 extra steps on the mitochondrial tree (fig. 4). Constraining these species to the positions indicated by the mitochondrial data requires five extra steps in the morphological characters. However, these morphological characters are highly homoplastic at the genus or family level, and thus it is not unreasonable to assume convergence within the genus *Greya*. There are two apparent examples in our data.

Uncertainty in the placement of *G. variata* in the morphological phylogeny is caused by two derived character states shared with the *solenobiella* group, the tapering of the cucullus beyond the pollex (character 22) and the reduction in segments in the maxillary palpus (character 2). Shape modification of the cucullus is a common trend in Lepidoptera, and the reduction of maxillary segments is homoplastic in the Prodoxidae, as discussed above.

The MP morphological tree indicates two synapomorphies uniting *G. pectinifera*, *G. politella*, *G. variabilis*, and *G. enchrysa* (fig. 1). Character 18, the shape of the basal costa of the valva, is homoplastic within *Greya*, as it is found in the derived condition in *G. obscura*. Character 25 is an unordered character (number of sensilla) which is highly variable within prodoxid genera.

The basal position of *G. pectinifera* and *G. variabilis* is, however, supported by several characters: they share a single, plesiomorphic genital character, a pectinifer (or modified pectinifer) on the cucullus of the male (character 19). *Greya pectinifera* has a pectinifer (a row of spines), while *G. variabilis* shows an intermediate state between a full pectinifer and reduction of this structure to a pollex (a single spine), which is the derived state shared by all other *Greya*. *Greya pectinifera* also has a scaly haustellum, a plesiomorphic trait shared only with the basal prodoxid genus *Tridentaforma*.

All three basal species in the mitochondrial phylogeny also show levels of sequence divergence from all other congeners, that are equal to or greater than sequence divergences among genera in the family Prodoxidae (J. Brown, unpublished data); under the assumption of a clocklike accumulation of base substitutions, this suggests that these are old lineages within
the genus. Thus, it would not be surprising if they have evolved features convergently with other *Greya* species, particularly in characters that show variation among other lineages in the family with the same degree of mtDNA divergence. In light of the congruence between the mtDNA evidence and a subset of morphological characters, we conclude that the mtDNA MP tree accurately places these three species as basal lineages.

**Solenobiella Group**

The mtDNA phylogeny indicates a sister relationship between *G. reticulata* and *G. subalba*, while the morphological data unites *G. reticulata* and *G. powelli*, on the basis of a single shared loss (figs. 1 and 4). Neither data set provides substantial enough support for relationships within this group to prefer either hypothesis. Both data sets support the sister relationship of *G. suffusca* and *G. solenobiella*. The widespread species *G. solenobiella* is paraphyletic with respect to mtDNA haplotype, as the *G. suffusca* haplotype falls within the *G. solenobiella* clade as sister to the *G. solenobiella* haplotype from the same location (Sequoia National Park in Tulare County, Calif.).

**Punctiferella Group**

The mtDNA phylogeny places *G. mitellae* as sister to one haplotype of *G. piperella*, rather than to *G. piperella* + *G. punctiferella*, as is indicated by the morphological data (fig. 4). The former topology requires an extra step in morphological character 4 (a reduction of the interantennal suture), while the latter topology requires seven extra mitochondrial substitutions.

**Greya obscuromaculata**

This species was placed as sister to the *punctiferella* or *solenobiella* groups in the morphological or mtDNA MP trees, respectively (fig. 4). However, when *G. pectinifera* and *G. variabilis* are placed in basal positions (as discussed above) three morphological synapomorphies unite *G. obscuromaculata* with the *punctiferella* group. While this topology requires a single extra step for the mtDNA data, positions supporting this node are relatively conservative (two, two, and four changes over the entire tree), compared with the characters that support unifying *G. obscuromaculata* with the *solenobiella* group (seven, eight, three, and three changes). In this case, morphological characters provide support for relationships for which the molecular data are ambiguous.

### Species-Group Relations

The relative positions of the *politella* and *punctiferella* species groups were reversed in the morphological and mitochondrial analyses. In each case, however, the groups could be switched with only a single-step increase in overall tree length. When *G. variabilis* and *G. pectinifera* are moved out of the *politella* group (as defended above), there is no difference in morphological tree length, with each grouping supported by a single synapomorphy. The lack of a large number of synapomorphies, in either data set, linking any two of the species groups, as well as equal average sequence divergence between the groups, suggests that the three lineages diverged within a short period of time, leaving few clues as to the order of divergence.

Attempts to confirm the branching order of these groups in the molecular phylogeny by using various forms of character and substitution weighting produced mixed results; character weighting supported the *politella* group as sister to the clade uniting the other two species groups (i.e., the mtDNA MP tree), while substitution weighting supported either topology (i.e., the mtDNA or morphological MP trees), depending on the relative weights given to transversions and transitions. Scrutiny of the mtDNA characters supporting the alternative topologies also indicates that the *politella* group is sister to a *punctiferella* + *solenobiella* group clade: this topology is supported by substitutions in four conservative characters (nucleotide positions) uniting the *punctiferella* and *solenobiella* groups (one, two, three, and four substitutions over the entire tree), the two most conservative of which occur at first and second codon positions. In contrast, substitutions uniting the *politella* and *solenobiella* groups are all at third-base positions in codons (two, three, and four substitutions over the entire tree). On the basis of this evidence, we conclude that the *politella* group is sister to a clade uniting the other two species groups.

In the above analyses, we have chosen to compare the support for different hypotheses of relationship (i.e., monophyletic groups) by comparing the morphological and molecular evidence for conflicting hypotheses. Kluge (1989) and Jones et al. (1993) argue persuasively against the use of taxonomic congruence or the comparison of hypotheses generated from different classes of data. The problems with the use of consensus cladograms derived from different data sets are well known (Kluge 1989; Swofford 1991). Kluge (1989) instead advocates the use of total evidence (morphological, molecular, behavioral, etc.) as a logical extension of the cladistic principle of the maximization of character congruence. While our conclusions are not altered if we use a total evidence MP tree, we agree with Swofford (1991) that much can be learned by comparing inferences from logically independent data sets (e.g., morphological data and mitochondrial sequence data or sequence data from unlinked genes involved in unrelated biochemical path-
The Evolution of Host Association and Mutualism

The phylogeny of Greya host associations clearly does not support a model of long-term cospeciation (association by descent) between moth species and their hosts. Rather, host shifts between distantly related families (e.g., Umbelliferae and Saxifragaceae) appear to have played an important role in speciation and diversification in the genus. This does not, of course, rule out coevolution between moths and their hosts. While these subjects are treated in detail elsewhere (Pellmyr and Thompson 1992; J. N. Thompson and O. Pellmyr, personal communication), we discuss below several general conclusions about the evolution of ecological traits and their importance in diversification in Greya.

Species in the solenobiella group are all associated with plants in the Umbelliferae, while all other species for which data are available are associated with Saxifragaceae. Although verification of hosts for the basal species may alter this view, the most parsimonious explanation of the evolution of host association at the family level proposes a switch from feeding on saxifrages to umbellifers in the common ancestor of the solenobiella group. This switch is associated with significant morphological change.

Species in the punctiferella group are known to feed on meristematic tissue as larvae, in contrast to members of the other two species groups, which are seed parasites. Although the larval biology of the basal species is unknown, the MP reconstruction of larval feeding mode supports a switch from seed parasitism to feeding on meristematic tissue in the common ancestor to the punctiferella group. As in the solenobiella group, this ecological shift is associated with significant morphological change.

The politella group contains the only two species that are known to pollinate their host plants during oviposition into the flower. Lack of biological information on the basal species prevents us from drawing a conclusion as to whether oviposition in the flower and pollination are plesiomorphic traits or whether they have evolved convergently in Greya and other genera in the Prodoxidae. Oviposition in the flower is considered plesiomorphic for the Agavaceae-feeding genera (Pellmyr and Thompson 1992). Its presence in a relatively basal species group in Greya, the sister group to the Agavaceae-feeding genera (as well as in the relatively more basal genus Tetragama: Pellmyr and Thompson 1992), raises the question whether this character, which is crucial to the evolution of mutualism between yucca moths and yuccas, may be older than the association of prodoxids with the Agavaceae.

The molecular phylogeny also indicates the potential importance of host shifts in speciation in Greya. As noted earlier, the G. suffusca haplotype is most closely related to a single G. solenobiella haplotype from the same locality. Since G. suffusca is known only from populations in this county, the mtDNA phylogeny strongly supports a recent origin of G. suffusca via a host shift from Yabea microcarpa to Osmorhiza brachypoda (table 1). A similar result is found in the punctiferella group, where the G. mitellae haplotype is most closely related to a single haplotype of G. piperella from northern California, making G. piperella paraphyletic with respect to mtDNA (fig. 2). It is interesting that the California population of G. piperella is associated with Heuchera micrantha rather than with H. cylindrica, the host for all other known G. piperella populations in eastern Oregon, Washington, and southern British Columbia; the California host species is available but not utilized by non-California populations. The California population of G. piperella is also aberrant in wing coloration and male genital shape characters (Davis et al. 1992), but the haplotype relationships do not by themselves justify separate species status for California G. piperella. As in the solenobiella group, there is an association of host shifts with morphological change. However, we view these results cautiously, as a lack of adequate population sampling makes it impossible to rule out lineage sorting from a polymorphic ancestor as an explanation of this pattern of haplotype relationships. Lineage sorting might also account for the relationships of populations in other species groups (e.g., G. solenobiella + G. suffusca) that are characterized by a level of sequence divergence less than that found within some widespread species.

Variation in Substitution Frequencies in the Cytochrome Oxidase Genes

The discovery of strong transition bias in prodoxid moth mtDNA is consistent with observations from several groups of closely related Drosophila species (DeSalle et al. 1987; Liu and Beckenbach 1992; Tamura 1992). The proportion of transitions is lower in comparisons of more distantly related taxa, presumably a consequence of the "dominance" of transversions at multiply substituted sites (Brown et al. 1982; DeSalle et al. 1987). In our data, transitions from A and T to G and C were approximately four times as common as transitions from G and C to A and T. Tamura (1992, table 4) found a similar bias in a study of nucleotide substitutions in nine species of Drosophila. This is perhaps a reflection of ob-
served A+T compositional biases rather than a cause. The observed purine:pyrimidine strand-specific bias in number of transitions (T$\leftrightarrow$C $>$ A$\leftrightarrow$G) also has been seen in mtDNA from *Drosophila* (Wolstenholme and Clary 1985; Garesse 1988; Tamura 1992) and mammals (Brown and Simpson 1982; Brown et al. 1982).

The proportion (80%) of A$\leftrightarrow$T substitutions among transversions is similar to that found among *Drosophila* species (Wolstenholme and Clary 1985; DeSalle et al. 1987; Tamura 1992). The majority of transversions in the prudoxid comparison occur at third-base positions in codons, where 90% of the bases are A and T. Extreme bias in base compositions appears to be typical of the mtDNA COI gene in insects; in 13 species representing 10 different orders, the mean proportion of A+T at third-base positions in the COI gene is 87% (Liu and Beckenbach 1992). The bias toward A$\leftrightarrow$T transversions could be the result of an underlying bias in the mutational spectrum or of selection (constraint) on base composition (or both). The bias in base composition is greatest at third-base positions (true for most orders of insects; Liu and Beckenbach 1992), perhaps because first- and second-base positions are more constrained by the amino acid composition of the encoded protein. This assumes that there is continuous selection for A+T nucleotides, which is opposed by selection against some nonsynonymous substitutions which incrase the representation of A's or T's but have deleterious consequences for protein function (see arguments in Wolstenholme and Clary 1985; DeSalle et al. 1987).

**Character and Substitution Weighting in Molecular Phylogenies**

How and when characters or types of character changes should be weighted during phylogenetic reconstruction is an unresolved and hotly debated issue (for recent examples, see Fitch 1992; Hedges and Maxson 1992; Marshall 1992). While it is reasonable to assume that heavier weighting of more conservative characters or changes should result in more accurate reconstruction of phylogeny, it is unclear how sensitive phylogenetic reconstruction is to different assumptions about character and substitution weights.

We submitted our mtDNA sequence data to several of the proposed methods of character and substitution weighting. Heavier weighting of first- and second-codon-position characters did not change our inferences from those based on equal weighting of all characters. Nodes joining clades with greater than 9% estimated sequence divergence were sensitive, however, to different assumptions about substitution weights. Ignoring transitions resulted in little phylogenetic resolution, indicating that transversion parsimony only resulted in the elimination of information at this level of divergence (DeSalle 1992).

Our data illustrate several problems with the use of inferred substitution frequencies for phylogenetic weights. First, estimates of substitution frequencies, in particular those of quickly accumulating transitional substitutions, are dependent on the ability of the phylogeny to reconstruct those changes. For example, estimates of transition bias dropped markedly when we included taxa with long branches along which transitions would be obscured by transversions (fig. 3). Second, algorithms that weight substitutions inversely with their frequency (e.g., dynamic weighting; Williams and Fitch 1989, 1990) are sensitive to base composition. For example, the 4:1 bias in A$\rightarrow$G and T$\rightarrow$C substitutions over their reciprocals in our data can be explained by the 80% A+T bias over the entire molecule.

Finally, conversion of substitution frequencies into phylogenetic weights often results in weight matrices that violate the triangle inequality (present study; A. Brower, personal communication). This is caused by the extreme rarity of certain substitutions; in our data, for example, A$\rightarrow$T, A$\rightarrow$G, and C$\rightarrow$T substitutions are common, while all other substitutions are extremely rare, leading them to be assigned maximum weights (tables 4 and 5). Maddison and Maddison (1992) point out that such matrices may not be self-inconsistent if the values reflect the probability of change over a certain period of time, i.e., over short branches. However, since these weights are normally applied to all branches regardless of length, inconsistency is not avoided by this assumption. The application of such weights may actually decrease the accuracy of phylogenetic inference by accentuating the attraction of long branches. Consider the following heuristic example: Only substitutions between A$\rightarrow$T, A$\rightarrow$G, and C$\rightarrow$T occur. Substitution frequencies are estimated by reconstruction on a phylogeny such as that of *Greya*, which contains areas of short branches and internodes, as well as long branches of basal lineages. Substitutions other than the six allowed are inferred, since multiple substitutions may occur on long branches. These substitutions are infrequent and are thus given heavy weight for phylogenetic reconstruction. If these substitutions occur in parallel along two long branches, application of weighted parsimony will strongly support uniting these long branches, so that two costly substitutions will be reduced to a single substitution.

More fundamentally, it is unclear why substitution type should take precedence over character type. For example, a transition at a second codon position may be more conservative phylogenetically than a transversion at a fourfold-degenerate site. Dynamic weighting (Williams and Fitch 1989, 1990) compensates for this problem by implementing both character and substitution weights. Fitch and Yc (1992) present a small set...
of simulations that indicate that dynamic weighting increases the accuracy of reconstruction in some cases. Strong evidence that this method or other iterative methods accurately balance the trade-off between character and substitution weights requires more extensive simulations.

**Sequence Availability**

Sequences are available from GenBank under accession numbers L22220–L22245.

**Acknowledgments**

We wish to thank two anonymous reviewers for critical comments that improved the manuscript. J.M.B. and R.G.H. thank the following people for technical help: Steve Bogdanowicz, Ben Normark, and Andy Brower. O.P. and J.N.T. thank the following people for logistic help: Don Frack, Paulette Bierzychudek, Susan Mazer, Jerry Powell, Nelsa and the late Buck Buckingham, Hal Hansel, and Wayne Wehling. The Nez Percé tribe of Idaho permitted collection of samples from their land. This work was supported by NSF grant BSR 8817337.

**APPENDIX**

Table A1

**Morphological Character Matrix for *Greya***

<table>
<thead>
<tr>
<th>Species</th>
<th>Characters (1–28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ancestral state</td>
<td>0000000000010000010000010000000000</td>
</tr>
<tr>
<td><em>G. punctigerella</em></td>
<td>100011001110010103001110011101001</td>
</tr>
<tr>
<td><em>G. piperella</em></td>
<td>10001100111001010300111101101001</td>
</tr>
<tr>
<td><em>G. mitellae</em></td>
<td>0000011001100101030011101101001</td>
</tr>
<tr>
<td><em>G. obscura</em></td>
<td>1000011000010210003000013000</td>
</tr>
<tr>
<td><em>G. obscuramorulata</em></td>
<td>100001100011071003000013000</td>
</tr>
<tr>
<td><em>G. politella</em></td>
<td>100001000001101010010300014111</td>
</tr>
<tr>
<td><em>G. enchyrsia</em></td>
<td>100001000011101011010300014011</td>
</tr>
<tr>
<td><em>G. variabilis</em></td>
<td>10001001000101101101201001010101010101010101</td>
</tr>
<tr>
<td><em>G. pectinifera</em></td>
<td>100010000122010100100300101200001</td>
</tr>
<tr>
<td><em>G. variata</em></td>
<td>100010000110000103000300101200001</td>
</tr>
<tr>
<td><em>G. subalba</em></td>
<td>111001100111000300101200001</td>
</tr>
<tr>
<td><em>G. solenobiella</em></td>
<td>111001100110100300101200001</td>
</tr>
<tr>
<td><em>G. suffusca</em></td>
<td>111001100110100300101200001</td>
</tr>
<tr>
<td><em>G. reticulata</em></td>
<td>111001100110100300101200001</td>
</tr>
<tr>
<td><em>G. powelli</em></td>
<td>111001100110100300101200001</td>
</tr>
</tbody>
</table>

**Note.** —Matrix is taken from Davis et al. (1992), with the addition of data missing at the time of publication. All characters were ordered (Wagner parsimony), with the exception of characters 13, 14, and 25, which were unordered (Camin-Sokal parsimony). For ordered characters, "0" is the plesiomorphic condition. A "9" indicates information that is not applicable and which was considered as missing in the phylogenetic analysis.

**Definition of Characters**

In the following list, p = plesiomorphic condition for ordered, multistate characters.

1. Male (0) not larger or (1) larger than female.
2. Maxillary palpus (0) of five segments or (1) three or four segments.
3. Interantennal suture (0, p) convex in dorsal view, (1) straight or concave, or (2) absent.
4. Interantennal suture (0) present or (1) reduced to a series of parallel ridges.
5. Vestiture of frons (0) dense or (1) sparse.
6. Metasternal furca (0) free or (1) fused to secondary arms.
7. Wing pattern (0) uniform or (1) sexually dimorphic.
8. Zigzag wing pattern (0) absent or (1) present.
9. Ground color of forewing (0) made up of uniform-colored scales or at least patches of uniform scales or (1) a result of pointillistic mixing of fuscous and white scales.
10. Wing pattern made up of (1) minute brown spots or (0) otherwise.
11. Cubitals in hindwing (0) parallel or (1) convergent.
12. Uncus (0) deeply bilobed, (1, p) shallowly bilobed, or (2) more or less pointed, unilobed.
13. Vinculum-saccus (0) short, (1) long, or (2) very long (unordered).
14. Number of cornuti (unordered).
15. Aedeagus (1) broad or (0) not apparently broadened at posterior end.
16. Aedeagus appears (0) undivided or (1) two pronged at anterior end.
17. Aedeagus (0) without or (1) with two finlike structures at apical end.
18. Basal costa on valva ends (0) round or (1) squarely against costal margin at notch. In all species a smaller or larger notch is present on the costa.
19. Cucullus with (0) no armature, (1, p) pectinifer, (2) short pectinifer or single spine (intraspecific variation), or (3) pollex.
20. Pollex (0) simple or (1) trifid in outer part.
21. Pollex (0) as melanized as adjacent areas or (1) strongly melanized.
22. Cucullus (1) constricted beyond pollex or (0) not.
23. Pollex in (0) middle or (1) outer half of valva.
24. Ovipositor (0) round or dorsoventrally flattened or (1) laterally flattened.
25. Caudal margin of seventh abdominal segment in female with different sets of long sensilla: (0) 20–25 sensilla on sternite, no sensilla on tergite; (1) 20–40 sensilla on sternite, 10–20 equally long sensilla on tergite; (2) 3–6 long sensilla along margin, shorter sensilla off margin on sternite, 2–7 moderately long sensilla on tergite; (3) 3 or 4 sensilla laterally on sternite, about 5 shorter sensilla on tergite; (4) 10–20 moderately long sensilla near or on margin on sternite, 8–15 short sensilla on or near margin on tergite; or (5) no sensilla on either plate (unordered).
26. Eighth abdominal segment in female (1) telescoping or (0) not.
27. Signa (0) present or (1) lost in corpus bursae.
28. Anterior portion of ductus bursae (0) membranous or (1) rugose.
LITERATURE CITED


POWELL, J. A., and R. A. MACKIE. 1966. Biological interrelationships of moths and Yucca whipplei (Lepidoptera:

Ramirez, B. W. 1970. Host specificity of fig wasps (Agaoni-


Tamura, K. 1992. The rate and pattern of nucleotide substitu-

Thompson, J. N. 1982. Interaction and coevolution. Wiley-
Interscience, New York.

———. 1987. Variance in number of eggs per patch: ovipo-


WiesJ, T. 1979. Coevolution of figs and their insect pol-


Thomas Eickbush, reviewing editor

Received April 12, 1993

Accepted August 26, 1993