Molecular Phylogeny Within and Between Species of the *Archips argyrospila* Complex (Lepidoptera: Tortricidae)

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ABSTRACT The *Archips argyrospila* complex consists of a series of morphologically indistinct populations with a confusing array of pheromone and host types. No phylogenetic studies on the genus *Archips* or any of its species groups have previously been published. We examined DNA variation in a 475 bp segment of the mitochondrial COI gene and compared 30 populations of *A. argyrospila*, four populations of *A. mortuana*, four populations of *A. goyerana*, and one specimen of each of four outgroup species. Among the 97 ingroup sequences obtained, there were 23 unique haplotypes and nucleotide variation at 20 sites. The monophyletic ingroup containing *A. argyrospila*, *A. mortuana*, *A. goyerana* and a West Coast clade was strongly supported by bootstrap values and decay indices. Host races and some pheromone types within *A. argyrospila* did not correspond to mtDNA variation in this analysis. Our molecular analyses supported phylogenetic and ecological interpretations of species status applied to *A. goyerana* but not *A. mortuana*, and supported the existence of a phylogenetically distinct West Coast species.

KEY WORDS Tortricinae, Archipini, *Archips argyrospila* host race, pheromone race, COI

Archips (HÜBNER 1822) is a widely distributed genus in the Holarctic, especially in the Palaearctic and Oriental subregion. In North America it is represented by at least 24 species. These species are distributed largely in the northern and central parts of the subregion but reach southward as far as Florida, Texas, and southern California (Razowski 1997). The fruittree leafroller, *Archips argyrospila* (Walker) is a widespread polyphagous pest on orchard trees throughout the northern United States and southern Canada (Chapman and Lienk 1971, Smirle 1993). Prentice (1965) reports that *A. argyrospila* occurs throughout southern Canada, but in the western provinces it ranges north to the 60th parallel. This moth is found in all except extremely arid regions and high elevations west of the 100th meridian (Chapman and Lienk 1971), ranging essentially from coast to coast.

Larvae of *A. argyrospila* have been reported in damaging numbers on almost all types of orchard crops and have been known to locally defoliate a wide variety of angiosperm forest tree species and deciduous shrubs (Powell 1964, Madsen and Morgan 1970, Chapman and Lienk 1971, Coates et al. 1989). Chapman and Lienk (1971) suggested that the original hosts of *A. argyrospila* in the northeast were various species of oak (*Quercus*), hawthorn (*Crataegus*), and birch (*Betula*). Powell (1964) stated that various *Quercus* species are the most common hosts in California. After the widespread planting of apple in North America, explosive outbreaks of *A. argyrospila* took place in Colorado, Montana, and some orchard districts of the Pacific Northwest between approximately 1890 and 1925 (Chapman and Lienk 1971).

The *A. argyrospila* complex consists of a series of morphologically indistinct populations, some formally named. The variability in appearance among individual *A. argyrospila* adults is remarkable, as noted by several authors (McDunnough 1923, Powell 1964, Razowski 1977). McDunnough (1923) thought there were several forms grouped around typical *A. argyrospila*, agreeing closely in general type of maculation and showing no decided differences in male genitalia. Two species descriptions within the complex were based largely on wing characters (*A. vividana* Dyar and *A. columbiana* McDunnough). Neither withstood subsequent morphological analyses, and both were synonomized by Powell (1964).

Variability in food plant preferences, pheromones, and even pesticide resistance among populations of *A. argyrospila* is well documented (Coates et al. 1989, Cossentine and Jensen 1991, Deland et al. 1993, Judd et al. 1993, Smirle 1993). MacKay (1962) and Goyer et al. (1995) suggested that *A. argyrospila* consists of a number of different sibling species. Goyer et al. (1995) tested pheromones and food plant preference, and MacKay (1962) separated three series of larvae within *A. argyrospila* based on their larval morphology. Their findings suggest that *A. argyrospila* is actually a closely related complex of species, which no one has attempted to examine systematically.

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Many forms initially appear compatible with species descriptions based on the ecological species concept (Van Valen 1976). The ecological species concept defines a species as a lineage that evolves separately from all lineages outside its range and that occupies an adaptive zone minimally different from that of any other lineage in its range. The independent evolution of lineages may be demarcated by food plant differentiation, thereby facilitating the development of host races or host-differentiated species (Legge et al. 1996, Bush and Smith 1997). Indeed, a few described species in the *A. argyrospila* complex are thought to be host specific and are identified largely by food plant preference and habitats (*A. myricana* McDunnough 1923, *A. eleagnana* McDunnough 1923, and *A. goyerana* Kruse 2000). *Archips mortuana* Kearfott owes its current species status to significant pheromone difference (Roelofs 1969) and other ecological characters (Chapman and Lienk 1971). However, there is little morphological evidence for such a designation (Kearfott 1907, MacKay 1962, Powell 1964, Razowski 1977).

Phylogenetic species (Eldredge and Cracraft 1980, Nelson and Platnick 1981, Mishler and Donoghue 1982, Nixon and Wheeler 1990, Cracraft 1997) may be grouped according to the possession of diagnostic characters, whether they are the autapomorphies of a monophyletic group or simply a diagnostic character combination. These are then ranked according to criteria such as the amount of character support for the group (Mishler and Brandon 1987). Allopatric groups should receive species status if the extent of their differences are similar to the average of related sympatric or parapatric sister species pairs rather than the minimum differences of such pairs (Sperling 2001). Ranking criteria used to assign species rank to diagnosable groups (Cracraft 1997) or gene clusters (Mallet 1995) may include ecological criteria or the presence of breeding barriers in particular cases. Ecological criteria, including pheromone data and host specificity, may be used to support the formal description of diagnosable groups determined through molecular analyses (Kruse 2000).

Despite wide interest in *A. argyrospila* and related species as pests, very little systematic research has been attempted on the complex. A confusing array of pheromone and host types contrasted with practically invariant genitalia make *A. argyrospila* an intriguing species for molecular investigation. The utility of mitochondrial DNA sequence analyses in systematic studies at the species level has been demonstrated in recent studies within the family Tortricidae (Sperling and Hickey 1994, Newcomb and Gleeson 1998, Landry et al. 1999). No phylogenetic studies on the genus *Archips* or any of its species have yet been published. This study examines mitochondrial DNA evidence of relationships within the morphologically similar, but often ecologically and behaviorally diverse, lineage currently known as *A. argyrospila.*

Materials and Methods

Specimens. The specimens used in this study were provided by collaborators or were collected by the authors. We selected 135 specimens for study, 112 *Archips argyrospila*, 10 *A. mortuana*, 9 *A. goyerana*, and 4 representing outgroups. Outgroup taxa included *Argyrotaenia niscana* Kearfott from Santa Barbara County, CA, (Landry et al. 1999), *Clepsis peritana* Clemens from Fairfax County, VA, *Archips purpurana* Clemens from Woodland County, IL, and *A. nigriplagana* Franclemont from Wilson County, TN. Outgroups were chosen on the basis of presumed close relationship with *A. argyrospila* (*A. nigriplagana*), presumed distant relationship but in the same genus (*A. purpurana*), and genera within the same tribe (*Argyrotaenia* and *Clepsis*) (Kruse & Sperling, unpublished data). A summary of successfully amplified ingroup material that we examined (97 of 131 specimens) is provided in Table 1, and a map of the distribution of samples is provided in Fig. 1.

Samples of the *Archips argyrospila* complex represented an array of forms differentiated by pheromone or food plant (Table 2). Specimens came from various sites representing a large portion of the range of *A. argyrospila* (Fig. 1). Where possible, we sampled at least three specimens from each locality to determine the extent of sequence divergence within populations. Specimens were collected using lights (UV, mercury vapor, or incandescent), searching foliage, or using commercially available pheromone (Scenturion catalog #168, Clinton, WA, and Gempler's Trécé FTLR, catalog #T3117, Belleview, WI). Live specimens were either frozen at –20°C, –70°C, or dropped directly into 95–100% EtOH. Pinned museum specimens were used to supplement this study when possible. The oldest successfully amplified sample was collected in 1982 (Table 1).

Specimens were identified initially by phenotype, specifically forewing pattern before DNA extraction. *Archips mortuana* were determined by Jerry A. Powell (Essig Museum of Entomology, University of California, Berkeley [EMEC]; five specimens, two amplified), Michael Sabourin, St. Paul, MN (three specimens, two amplified), and J.J.K. (two specimens, two amplified). All *A. goyerana* specimens were determined by J.J.K. The abdomen and wings of each specimen were preserved in a gelatin capsule for confirmation of identification, and these vouchers were deposited in the EMEC.

Molecular Techniques. Total genomic DNA was extracted using a QIAamp DNA Mini Kit #51306 (Qiagen, Valencia, CA). Most amplified fragments were ≈400–500 bp long. Amplifications were performed on an Ericomp TwinBlock EasyCycler using a hot start: *Taq* was added at the end of an initial denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 45°C, 1 min at 72°C, and a subsequent 10 min final extension at 72°C. For many of the older museum specimens, amplifications were performed on an MJ Research PTC200 using a hot start: *Taq* was added at the end of an initial denaturation at 94°C, followed by 10 repe-

Table 1. Locality data for all ingroup specimens sequenced

Locality data	Haplotype Codes	No. of specimens	Collector, Year
Alberta, Demmitt Campground, NW of Hythe	Aa7	1	F. Sperling, 1998
Alberta, Edmonton	Aa11	1[a]	F. Sperling, 1998
Alberta, Edmonton	Aa7	2	J. Emond, 1989
Alberta, Kleskun Hills	Aa7	1[a]	F. Sperling, 1998
Alberta, Lethbridge	Aa7, Aa17	3	J. Emond, 1989
Alberta, Touchwood Lake	Aa7, Aa16	2	G. Pohl, 1995
Alberta, Zama	Aa7, Aa15	2	G. Pohl, 1997
British Columbia, Summerland Station	Aa2	2	C. Krupke, 1998
CA: Alameda Co., Berkeley	Aa1, Aa2	2	J. Powell, 1995, 1996
CA: Monterey Co., Big Creek Reserve	Aa2, Aa4	5	J. Kruse, 1998
CA: Monterey Co., Hastings Reserve	Aa2	1	J. Powell, 1997
CA: Riverside Co., San Jacinto Mts.	Aa2	3	J. Brown, 1986
CA: San Diego Co., MCAS Miramar	Aa2	3	N. Bloomfield, 1998
CA: Sierra Co., Sierraville	Aa3	7	J. Powell, 1995
CA: Solano Co., G.L. Stebbins Cold Canyon Reserve	Aa2, Aa3, Aa5	3	J. DeBenedictis, 1999
CA: Yolo Co., Davis	Aa3	3	J. DeBenedictis, 1998, 1999
CA: Yuba Co., Brushy Creek, near Marysville	Aa3, Aa6	3	J. Kruse, 1999
CO: Jefferson Co., Idledale	Aa7, Aa8	3	Unknown, 1994
IN: Elk Co., no further data	Aa7, Aa9, Aa10	3	Unknown, 1998
LA: Assumption Parish, Pierre Part	Ag1, Ag2	3	R. Goyer, 1999
LA: Bossier Parish, Barksdale Air Force Base	Aa7	1	D. Pollock, 1996
LA: St. Charles Parish, Norco	Ag1, Ag2	3	R. Goyer, 1999
MN: Anoka Co., Blain, Janes Field	Aa7	2[a]	M. Sabourin, 1995, 1996
MO: Taney Co., Mark Twain National Forest	Aa7	2	J. Powell, 1991
MS: Harrison Co., Long Beach	Aa7, Ag1	2	R. Kergosien, 1997
MS: Hinds Co., Clinton	Ag2, Ag3	2	M. & E. Roshore, 1996
NC: Brunswick Co., Bald Head Island	Aa11	4	J. Sullivan et al. 1994
Quebec: Gatineau, Lake Aylmer	Aa7	1	B. Landry, 1998
Quebec: LaSarre, Lake Abitibi	Aa9, Aa18, Aa19	5	B. Landry, 1998
Quebec: 30 mi. N. New Richmond	Aa7	2[a]	W. Middlekauff, 1983
Saskatchewan: Big River	Aa7, Aa9, Aa20	3	G. Pohl, 1995
TX: Harris Co., Houston	Aa7, Aa12	4	E. Knudson, 1999
WA: Chelan Co., Howard Flat	Aa2	1	P. Himmel, 1997
WA: Chelan Co., Wenachee	Aa2	2	P. Himmel, 1997
WA: Chelan Co., Mission Creek Road	Aa2	1	E. Lagasa, 1996
WA: Thurston Co., Tenino	Aa13	3	E. Lagasa, 1996
WI: Burnett Co., Grantsburg	Aa7, Aa9, Aa14	6	M. Sabourin, 1998

The number of specimens collected at each locality as well as collector and year are provided in columns three and four.
[a] Specimens identified as *A. mortuana* had haplotypes Aa7 and Aa11.

titions of 30 s at 94°C, 30 s at 40°C and 40 s at 72°C, 10 repetitions of 30 s at 94°C, 30 s at 45°C and 40 s at 72°C, and 15 repetitions of 30 s at 94°C, 30 s at 50°C and 40 s at 72°C, and a subsequent 3 min final extension. Polymerase chain reaction (PCR) products were cleaned using a QIAquick PCR Purification Kit #28106 (QIAGEN). The PCR product was cycle sequenced with Perkin-Elmer/ABI Dye Terminator Cycle Sequencing Kit with AmpliTaq FS (Perkin-Elmer/Applied Biosystems, Foster City, CA) on an MJ Research PTC200 according to Perkin-Elmer's suggested thermal profile. The sequenced product was filtered through Sephadex-packed columns and dried. This product was resuspended and electrophoresed on an Applied Biosystems International 377 automated sequencer. All fragments were sequenced in both directions. Sequences were aligned manually to the sequence of *Drosophila yakuba* Burla (Clary and Wolstenholme 1985).

We chose a 475 bp segment in the COI gene to compare specimens from 30 populations of *A. argyrospila*, four populations of *A. mortuana*, four populations of *A. goyerana*, and one specimen of each of the four outgroup species. This fragment corresponds to

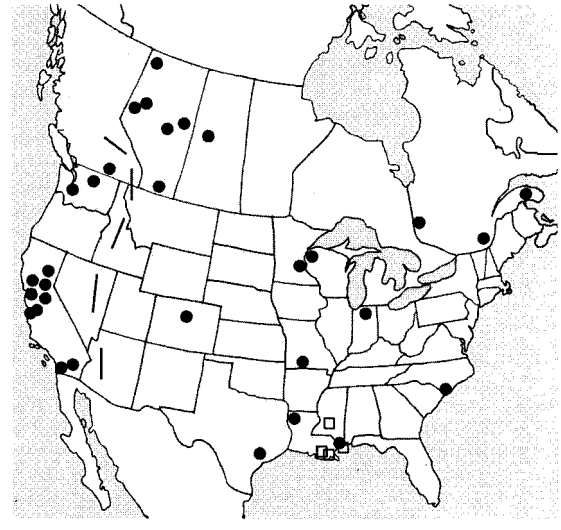

Fig. 1. Distribution of ingroup samples. Circles denote *A. argyrospila* and *A. mortuana*, squares denote *A. goyerana* sample sites. Dashed lines separate the West Coast Clade.

Table 2. In group populations that were examined in this study where pheromone was tested or an ecological difference from allopatric populations is known

	Species	Population	Biologial Difference (Source)
1	*A. argyrospila*	Thurston Co., WA	Pheromone differs from E. Washington populations (E. Lagasa, personal communication).
2	*A. argyrospila*	Burnett Co., WI	Attracted by Gempler's Trécé (catalog #T3117, Belleview, WI, USA) lure (M. Sabourin, personal communication).
3	*A. argyrospila*	Lethbridge, AB	Feed on Green Ash (*Fraxinus* spp.) (J. Emond, label data).
4	*A. mortuana*	Various	Pheromone differs from *A. agyrospila* (Roelofs 1969).
5	*A. goyerana*	Southern Louisiana	Feed on Baldcypress, and pheromone differs from N. Louisiana and California populations (Goyer et al. 1995).
6	*A. agyrospila*	Yuba Co., CA	All specimens collected using Scenturion© (catalog #168, Clinton, WA, USA) lure (JJK).
7	*A. argyrospila*	San Diego Co., CA	Moths apparently not attracted by Scenturion© (catalog #168) lure placed in suitable location during flight period (C. Conlan, personal communication).

near the middle of COI, between base pair numbers 2183 and 2659. Sequence was obtained by PCR amplification using the end primers CI-J-2183: 5′ CAA CAT TTA TTT TGA TTT TTT GG 3′ and CI-N-2659: 5′ GCT AAT CCA GTG AAT AAT GG 3′. *Archips purpurana* and *Clepsis peritana* required a different version of CI-N-2659: 5′ GAT AAT CCT GTA AAT AAA GG 3.

Phylogenetic Analyses. Analyses under parsimony were carried out using PAUP 4.0b4 (Swofford 1999). Sequence alignments were done manually, and no indels were found relative to *D. yakuba.* Variable nucleotide positions were treated as unordered characters with one state for each nucleotide. We employed a heuristic search with 1,000 random taxon addition sequence replicates (all branch-swapping algorithms found the same set of trees). Identical haplotypes were removed for the searches. To assess branch support, the equally weighted sequence data set was bootstrapped 1,000 times using a heuristic parsimony search with simple taxon addition sequence branch swapping. Decay indices were assessed by successively relaxing parsimony one step until the phylogeny collapsed into a polytomy. A neighbor joining tree was constructed from the data using the default parameters in PAUP 4.0b4 (Swofford 1999), with no topological constraints enforced. Sequences from *Argyrotaenia niscana*, *C. peritana*, *Archips purpurana*, and *A. nigriplagana* (all Archipini) were used to root the trees.

Results

Sequence Variation. We were able to obtain 475 bp of sequence for 97 of the 131 ingroup specimens selected for study (82 *A. argyrospila*, 6 *A. mortuana*, and 9 *A. goyerana*). Failed amplifications include all 8 specimens that were collected before 1982, 17 out of 30 specimens that were collected between 1982 and 1990, and 9 out of 93 specimens collected after 1990. No specimens preserved by freezing at −70°C or −20°C, placed alive into 95–100% EtOH, or recently field-pinned failed to amplify.

Among the 97 ingroup sequences obtained, there were 23 unique haplotypes, with nucleotide variation at 20 nucleotide sites (Table 3). The distribution of haplotypes varied among populations. For example, we found only one haplotype in seven specimens from Sierraville, but different haplotypes were found in each of three specimens from Elk County, IN, G. L. Stebbins Cold Canyon Reserve, CA, and Big River, Saskatchewan, Canada (Table 1). Sequence variation did not result in any amino acid replacements. All synonymous changes took place in the third codon position, with the exception of one first position change in the leucine codon at base number 2437 (Table 3).

Table 3. Nucleotide variation in 20 unique MtDNA COI haplotypes of *A. argyrospila* (Aa code numbers) and three of *A. goyerana* (Ag code numbers)

Haplotype code	Base Pair Numbers 22222222222222222222 12222333444455556666 83789235035627891134 75408548971904926910	No. of specimens	No. of localities
Aa1	CCATAATGATCTGCATGGTA	1	1
Aa2	........G...A.......	18	10
Aa3	....G...G...A.......	13	4
Aa4	........G..CA.......	2	1
Aa5	....G...G...A..C....	1	1
Aa6	....G...G.T.A.......	1	1
Aa7	T....G.........C..A.	25	17
Aa8	T....G......A..C..A.	1	1
Aa9	T.G..G.........C.AA.	7	4
Aa10	T....GA........C..A.	1	1
Aa11	T.............C..A.	5	2
Aa12	T....G.......T.C..A.	1	1
Aa13	........GC..........	3	1
Aa14	T....G........C...A.	3	1
Aa15	T....G........C..AA.	1	1
Aa16	TT...G.........C.AA.	1	1
Aa17	T....G.........C..AG	1	1
Aa18	T..........A..C..A.	1	1
Aa19	T....G....T....C..A.	1	1
Aa20	T....G.A......C...A.	1	1
Ag1	T....G.AG.TCAT.CAAA.	3	3
Ag2	T....G.AG..CAT.CAAA.	5	3
Ag3	T..C...AG..CAT.CAAA.	1	1

The number of specimens with each haplotype and the number of localities where each haplotype was found are given in columns three and four. Base pair numbering corresponds to homologous sequence in *Drosophila yakuba* Burla (Clary & Wolstenholme 1985).

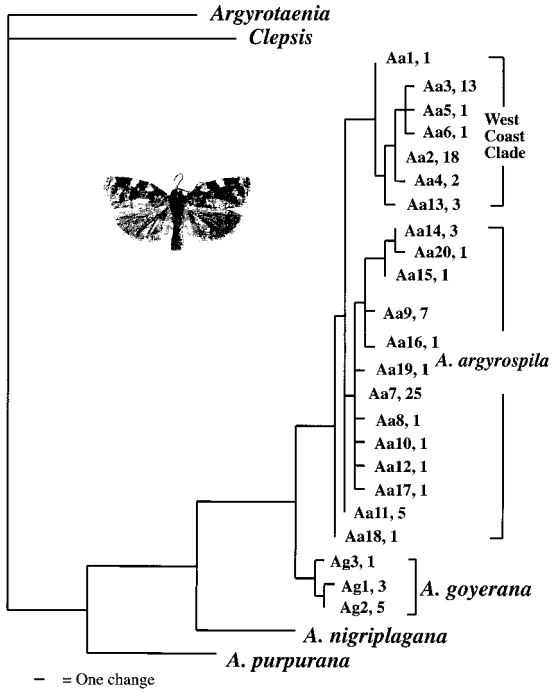

Fig. 2. Representative phylogram from 74 most parsimonious unrooted trees of 23 ingroup mtDNA COI haplotypes. *Archips argyrospila*, West Coast glade, and *A. goyerana* haplotype codes are followed by the number of specimens analyzed per haplotype. CI = 0.750, RI = 0.778, RC = 0.562.

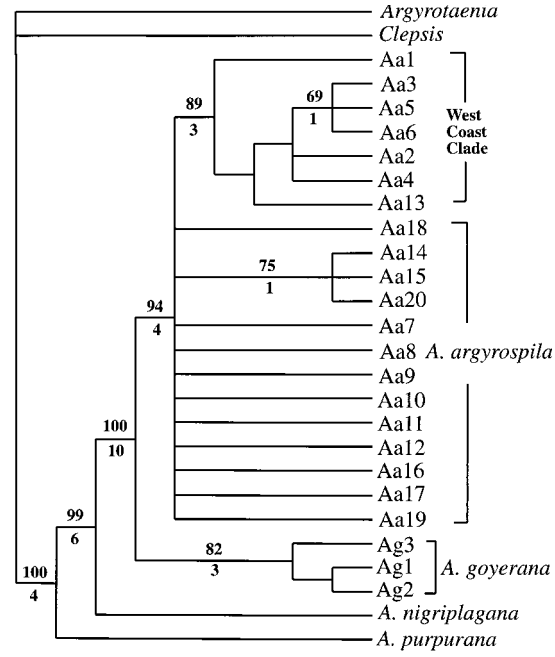

Fig. 3. Strict consensus of 74 most parsimonius unrooted trees of 23 ingroup mtDNA COI haplotypes. Numbers at branch nodes indicate bootstrap-decay index values. Only bootstrap values >50% are shown. CI = 0.750, RI = 0.778, RC = 0.562.

Of the 88 *A. argyrospila* and *A. mortuana* sequences of 475 bp, 20 haplotypes were unique. Of the haplotypes discovered, 40% were unique to single specimens. One haplotype (Aa7) was found in 17 different localities, and another (Aa2) in 10 different localities. Haplotypes Aa3 and Aa9 were found in four localities each, and haplotype Aa11 was found in two localities. The 15 remaining haplotypes were unique to single specimens from 14 localities (Lake Abitibi, LaSarre, Quebec, produced Aa18 and Aa19). Nine *A. goyerana* sequences of 475 bp revealed three unique haplotypes varying in two positions (Table 3). Two haplotypes were found in three out of the four localities sampled. Only one *A. goyerana* haplotype was unique to a locality.

Phylogenetic Analysis. A heuristic parsimony search of the 23 haplotypes in the *A. argyrospila* complex in PAUP 4.0b4 initially resulted in 74 trees of 128 steps (CI = 0.750, RI = 0.778, RC = 0.562). Included in the COI sequences were 47 parsimony-informative characters and 40 parsimony-uninformative characters. A representative phylogram is shown in Fig. 2. The exceptions to complete correspondence among phylograms include various basal placements of Aa11 and Aa18 and placement of the clade containing Aa14, Aa15, and Aa20, which was sister to the West Coast clade in half of the phylograms. A strict consensus tree of the 74 most parsimonious trees is shown in Fig. 3 and corresponds to the bootstrap consensus tree. A neighbor joining phylogram (Fig. 4) is provided to illustrate known biological differences described in Table 2 with all 97 ingroup operational taxonomic units.

The well-supported clades of the topology presented here (Figs. 2–4) were consistently derived in all analyses. The monophyletic ingroup containing *A. argyrospila*, *A. mortuana*, *A. goyerana*, and the West Coast clade was strongly supported by bootstrap values (100%) and decay indices (parsimony relaxed to 10 steps) as was the ingroup plus the closely related outgroup species, *A. nigriplagana* and *A. purpurana*. *Archips argyrospila*/*A. mortuana*, *A. goyerana*, and the West Coast clade were supported by bootstrap values of 94, 82, and 89%, respectively, and parsimony could be relaxed four steps before collapsing all of these portions of the cladogram into a polytomy (Fig. 3).

The West Coast clade was supported by a decay index of three and is diverged from the rest of *A. argyrospila* by 0.63–1.26%. This clade comprised 39 specimens and included seven haplotypes (Aa1, Aa2, Aa3, Aa4, Aa5, Aa6, and Aa13). Moderate bootstrap support (69%) was found for a subset clade of populations from the Pacific slope of the Sierra Nevada Mountains in California (haplotypes Aa3, Aa5, and Aa6). Similarly, a clade represented by three haplotypes (Aa14, Aa15, and Aa20, from Wisconsin, Alberta, and Saskatchewan, respectively) received moderate bootstrap support (75%). This clade was found associated with haplotypes Aa9 and Aa16 in half of the 74 most parsimonious phylograms and as a sister group to the West Coast clade in the other half.

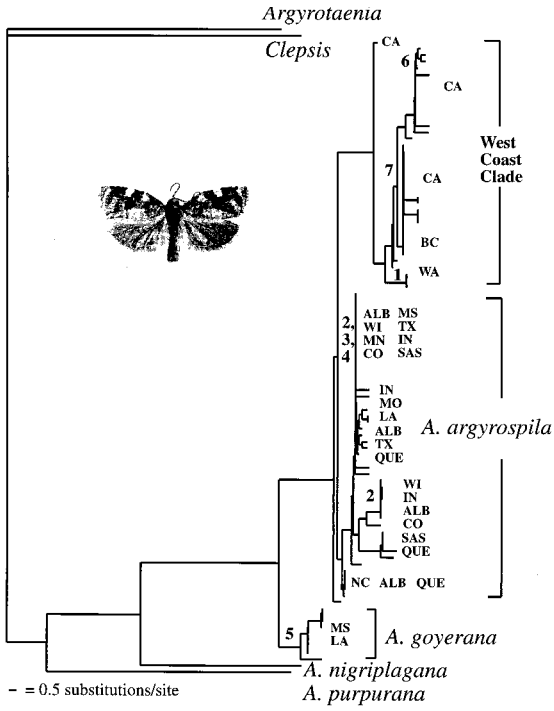

Fig. 4. Neighbor Joining phylogram of 97 ingroup mtDNA COI operational taxonomic units. Numbers at branch nodes or near operational taxonomic unit groups correspond to biological differences outlines in Table 2.

The mtDNA of specimens identified as *A. mortuana* did not show a pattern of relationships that supported separation of this taxon as a distinct species. The six specimens of *A. mortuana* that gave amplifiable DNA product were represented by two haplotypes: Aa7, the most common haplotype of *A. argyrospila*, and the geographically disparate haplotype Aa11 found in two localities (Brunswick County, NC, and Edmonton, Alberta). For the purposes of this article, specimens initially identified as *A. mortuana* are grouped with those of nominate *A. argyrospila* populations in further discussions.

Known pheromone and food plant differences among some populations of *A. argyrospila* and between *A. argyrospila* and *A. mortuana* (Table 2; Fig. 4) were not corroborated by molecular data. Although pheromone differences are known to exist between populations in eastern and western Washington, molecular data revealed only a four base pair difference (0.84% divergence). Where some moths in Wisconsin and California (Yuba County, CA) were attracted to pheromone lures, others were not (San Diego County, CA). The California populations were minimally different from each other, separated by less than two base pairs. Food plant difference was a good indicator of divergence when *A. goyerana* was compared with *A. argyrospila.*

Discussion

Assessment of *Archips argyrospila.* The topology illustrated in Figs. 2–4 reveals a monophyletic West Coast clade and eastern clades of *A. argyrospila.* Although McDunnough's (1923) interpretation of *A. columbiana*, described from Salmon Arm, British Columbia, is sufficiently broad to include all West Coast populations, the morphological characters that he examined are not consistently diagnosable. Recognition of this previously synonomized species to include the entire West Coast clade is supported by the phylogenetically distinct nature of its mtDNA variation. However, we decline to resurrect this species from synonomy at this time because it is not consistently diagnosable by nonmolecular means. Because good morphological characters are yet to be found, we think that extensive ecological study of West Coast populations are needed to justify species status, including further research on pheromone chemistry and food plant relationships. We have noted that certain western *A. argyrospila* are strongly attracted by Scenturion lures (Yuba County, CA), whereas others (San Diego County, CA) apparently are not, yet there is little difference between mtDNA COI sequences. Both eastern and western *A. argyrospila* populations feed on oaks, are often pests of orchard trees, and appear to be equally polyphagous.

Common eastern *A. argyrospila* haplotypes are found west to Jefferson County, Colorado, and northwestern Alberta, Canada. Regardless of the huge expanse of territory included in the range of *A. argyrospila*, no haplotype differs from any other by >0.84%. In comparison, molecular data indicates a divergence ranging from 1.47 to 2.53% between populations of *A. argyrospila* and *A. goyerana.* However, phenetic measurements, such as simple percent sequence divergence, is highly variable among closely related lepidopterans and is not necessarily a good predictor of species (Landry et al. 1999). For example, among three species of ermine moths (Yponomeutidae) <1% divergence occurred across the entire 2.3 kb COI+II region (Sperling et al. 1995), and sequence divergence between haplotypes from different populations of *Greya obscura* (Lep: Prodoxidae) ranged from <1% to as much as 5.7% across a 765 bp region of COI+II (Brown et al. 1994).

Archips mortuana posed unexpected difficulty. It was originally described as a variety (Kearfott 1907), but its identity according to the description is questionable. Specimens examined by Kearfott were from Ottawa, Canada; New Brighton, PA; Wisconsin (no further data); and San Francisco, CA; plus two additional specimens described as "badly rubbed" from Algonquin, IL, and south Utah. No holotype was designated. Other authors began to refer to *A. mortuana* as a species, apparently starting with Meyrick in 1913. Klots (1942) designated the New Brighton specimen as the lectotype and the Wisconsin specimen as a 'paralectotype'. Presumably, he examined the two rubbed specimens but either ignored or was unable to locate the Ottawa and San Francisco specimens. Free-

man (1958) perpetuated this information without comment. Powell (1964) synonomized *A. mortuana* under *A. argyrospila.* Roelofs (1969) used material identified as *A. mortuana* from the Hudson Valley and Geneva, NY, to conduct pheromone experiments. He found that they were not attracted to local *A. argyrospila* females and visa versa. These moths may have been equivalent to Kearfott's New Brighton lectotype, but not to California specimens. Powell recognized *A. mortuana* as a species in a checklist of the Tortricidae (Hodges et al. 1983) based on the pheromone studies by Roelofs (J. A. Powell, personal communication), whereas Razowski (1977) chose not to recognize *A. mortuana.* Questions remain as to what *A. mortuana* is and how to identify it.

Ecologically and Phylogenetically Distinct Species. The results of mtDNA analyses presented here support recognition of *A. argyrospila* and *A. goyerana* as presented in previous work (Goyer et al. 1995, Kruse 2000). *Archips goyerana* and *A. argyrospila* occur together where baldcypress (*Taxodium distichum* L. Rich.), the food plant of *A. goyerana,* and oak forests interface, and potentially also where baldcypress is planted as an ornamental. Extensive rearing studies by Goyer et al. (1995), including food plant switching and pheromone attraction, are strong support for *A. goyerana* as an ecologically distinct species.

There is interest in using ecological evidence, such as food plant and habitat, to denote undescribed species or populations worthy of protection under the Endangered Species Act or similar state law (Legge et al. 1996). Food plant and pheromone differences were instrumental in calling attention to *A. goyerana* initially (Goyer et al. 1995) and were invaluable data for the description of the species (Kruse 2000). Except for comparisons between *A. goyerana* and *A. argyrospila,* host race and pheromone types within *A. argyrospila* sensu lato did not correspond in a convincing way with mtDNA COI variation. Under the ecological species concept, the determination of a potential new cryptic species may be limited to characters such as host specificity versus polyphagy. In cases like the *A. argyrospila* complex, ecological character distinctions are evidence used to identify potential species, although several lines of evidence are needed to support a formal description.

The results of this study support the use of mitochondrial DNA sequence as a valuable marker for supporting the description of new species where morphological differences are extremely subtle, as was found in previous work (Sperling and Hickey 1994, Cognato et al. 1999). Molecular analyses in this study supported *A. goyerana* and the West Coast clade as phylogenetically distinct species. Ecological and morphological interpretations of species applied to *A. goyerana* provided additional characters necessary for its formal description (Kruse 2000). The West Coast clade is not yet supported as a species by other characters, and therefore we make no recommendation regarding taxonomic recognition at this time. Removal of the West Coast clade would leave *A. argyrospila* with paraphyletic mtDNA, but we do not consider the absence of strict monophyly to be an impediment to species recognition as long as it has a diagnostic character combination.

Archips mortuana was not distinct in molecular analyses despite having ecological support sufficient to rank it as a species. Kearfott's (1907) series of specimens examined for the description of *A. mortuana* included a specimen from San Francisco, locality data that we find questionable in light of the West Coast mitochondrial clade. We do not know for certain whether Kearfott's specimens from New York and the Hudson Valley, and from Alberta, Quebec, and Minnesota, are conspecific.

From Roelofs' (1969) work, we have extensive pheromone data in support of a sibling species based on pheromone chemistry. Whether his application of the name *A. mortuana* to it is valid has not been confirmed. Specimens that were determined by three separate persons as *A. mortuana* do not qualify as a species under the phylogenetic species concept in this study. Food plant associations of populations assigned to *A. mortuana* are not so restricted as that of *A. goyerana,* nor are they very taxonomically divergent from those of *A. argyrospila.* Thus, pheromone differentiation and host specificity documented for *A. goyerana* (Goyer et al. 1995) are supported by phylogenetic analysis of mtDNA. The results of this study support formal taxonomic recognition of *A. argyrospila* and *A. goyerana* as species. They also provide preliminary support for the existence of a phylogenetically distinct West Coast clade, but do not support the recognition of *A. mortuana.*

Sequence Availability. Sequences for outgroups, *A. argyrospila* (Aa1), and *A. goyerana* (Ag1) are available from GenBank under accession numbers AF308931 and AF309509-AF309513.

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