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SYSTEMATICS

DNA-Based Identification of Introduced Ermine Moth Species in North America (Lepidoptera: Yponomeutidae)

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Ann. Entomol. Soc. Am. 88(2): 155–162 (1995)

ABSTRACT Three sibling species of European small ermine moths of the *Yponomeuta padella* complex have been collected in North America. To allow identification of fresh as well as dried specimens without host data, we examined these three species for diagnostic differences in their mitochondrial DNA (mtDNA). We report the sequence for a 2.3-kb region of mtDNA that includes the cytochrome oxidase I and II and transfer RNA (tRNA) leucine genes. The mtDNA of *Y. malinellus* Zeller showed 0.8–0.9% divergence from that of the other two species, *Y. padella* (L.) and *Y. cagnagella* (Hübner). The latter two species had divergences of only 0.0–0.2%. A survey of restriction sites in 44 collections demonstrated that *Y. malinellus* has distinct, diagnosable mtDNA in North America, whereas *Y. padella* and *Y. cagnagella* were not distinguished from each other by this method. The recent establishment and range expansion of *Y. malinellus* and *Y. padella* in western North America is currently being monitored by pheromone traps that potentially have some degree of cross-attraction. Restriction sites in amplified mtDNA should allow rapid confirmation of species identities of such dried collections. The method used to generate the particular diagnostic reported here can be expanded readily to include new species and populations.

KEYWORDS *Yponomeuta malinellus, Yponomeuta cagnagella, Yponomeuta multipunctella*

IN EUROPE, SMALL ERMINE moths of the genus *Yponomeuta* Latreille constitute a well-studied group of nine species. Five of these species belong to the *Yponomeuta padella* complex and are characterized by their close morphological similarity, but show differences in host preferences and other biological traits (Menken et al. 1993).

Three species of this complex have been introduced into North America and have become pests. Their foliage-feeding larvae live communally in large webs, causing losses to fruit orchards and unsightly damage to ornamental shrubs. *Yponomeuta cagnagella* (Hübner) has been recorded from northeastern North America since the 1970s; it feeds on introduced species and varieties of *Euonymus* L. (Celastraceae) and is a cosmetic pest of these ornamental shrubs. *Yponomeuta malinellus* Zeller has been established in British Columbia and Washington since the early 1980s (Anonymous 1985); it feeds on *Malus* Miller and *Pyrus* L. (Rosaceae) (Menken et al. 1993) and is now a pest of apple orchards in western North America (Unruh et al. 1993). *Yponomeuta padella* (L.) has recently been found in southwestern British Columbia (Wood & Van Sickle 1994); it feeds on various woody Rosaceae (*Prunus* L., *Crataegus* L., *Sorbus* L., and *Amelanchier* Medikus) (Menken et al. 1993) and could become a pest of cultivated cherry and plum. Both *Y. malinellus* and *Y. padella* were recorded from numerous counties in New York during 1909–1912 (Parrot & Schoene 1912) but these early eastern introductions seem to have disappeared (Herrebout & Menken 1990).

Yponomeuta multipunctella Clemens is the only species in the genus that is native to North America and that is at all similar to the members of the *Y. padella* complex (Heppner & Duckworth 1983; J.-F.L., unpublished data). A fourth European species, *Y. plumbella* (Denis & Schiffermüller), has been recorded in North America from a single specimen collected in Massachusetts in 1949 (Hoebeke 1987). Both *Y. multipunctella* and *Y. plumbella* feed on species of *Euonymus*, but are easily distinguished from the members of the *Y. padella* complex by their wing coloration (Dyar 1900, Hoebeke 1987). Neither species is considered part of the *Y. padella* complex (Menken et al. 1993).

Although multidisciplinary studies have demonstrated their distinct status, the three introduced species of the *Y. padella* complex are extremely similar to one another in morphology and allozymes. Accurate identification of adults is still ultimately dependent on knowledge of the host associations of the larval stage, which is considered the most reliable means of confirming species identifications (Menken et al. 1993). Discriminant functions based on genitalia measurements have been developed for the separation of adults of these spe-

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0013-8746/95/0155–0162$02.00/0

cies but the proportion of specimens correctly identified to species ranges from 58 to 97%, depending on the sex of the specimen, characters available and the species distinguished (Povel 1984). Allozyme differences among the three species indicate a degree of genetic similarity that is normally found only among intraspecific populations (Menken 1989). Nonetheless, individuals from sympatric populations of the most similar pair of species, *Y. malinellus* and *Y. padella,* can still be identified by a complete allele substitution at one locus and show highly significant allele frequency differences for three other loci (Menken 1982, Arduino & Bullini 1985). Unfortunately, allozymes degrade easily and cannot be used to identify dried material (Menken & Ulenberg 1987). The species of the *Y. padella* complex also produce different sex pheromones (Löfstedt et al. 1991). Pheromone traps containing the primary chemical components are used to monitor populations (Unruh et al. 1993), but the possibility of a small degree of interspecific cross-attraction must be considered for such traps.

Consequently, there is a clear need for diagnostic characters that can be used to confirm identifications of dried adult material such as pinned museum specimens without host data and samples collected in pheromone traps. It is particularly important to be able to distinguish *Y. malinellus* and *Y. padella* because these two species currently cooccur in British Columbia and are being monitored to determine whether they will expand their range (Wood & Van Sickle 1994; E. LaGasa, personal communication). Pheromone trap data have contributed to decisions to variously restrict the movement of apple nursery stock in Washington, Oregon, and British Columbia since 1986 (E. LaGasa, personal communication). The presence of *Y. padella* in the same region implicates a different set of hosts, including cherry and plum, and underscores the need for diagnostic information that will allow more effective focusing of control strategies.

In this study, we attempted to develop a DNA-based means to identify the introduced species of the *Y. padella* complex in North America. For three reasons, we chose to survey mitochondrial DNA (mtDNA) for differences between these species. First, the mtDNA molecule occurs as multiple, relatively stable copies in each genome (Avise 1991), which should increase the chances of an accurate assay of degraded material. Second, mutation rates of mtDNA are relatively fast and have already allowed effective identification of lepidopteran races and sibling species (Bogdanowicz et al. 1993, Brower 1994, Brown et al. 1994, Sperling & Hickey 1994). Third, interspecific hybrids in Lepidoptera generally show more inviability or infertility in females than in males (Haldane 1922). Because mtDNA is inherited maternally, any tendency toward differential elimination of female interspecific hybrids should make mtDNA a good marker of species limits (Sperling 1993).

We sequenced a 2.3-kb region of mtDNA from one unambiguously identified, representative specimen of each of the three introduced species, in an effort to find sequence differences that could be assayed in small fragments amplified from degraded DNA. We then tested potential diagnostic restriction sites and surveyed for differences in material collected across the current ranges of these three species in North America. To clearly establish species identities, we restricted our survey to reared, wild-collected specimens for which the larval host had been identified. Our results show that three small fragments of amplified mtDNA can be used to separate dried specimens of *Y. malinellus* from *Y. padella* and *Y. cagnagella,* as well as to identify the more distantly related native species, *Y. multipunctella.* Because of the close similarity of the mtDNAs of *Y. padella* and *Y. cagnagella,* the method does not currently distinguish these two species from each other.

Materials and Methods

We began our search for potential diagnostic differences by sequencing a relatively large region of mtDNA in one specimen of each species. Identification was based on larval host plant, which is considered the best character for distinguishing these species (Menken et al. 1993). We reasoned that it would be easier to find restriction site differences between the very similar mtDNAs that such sibling species were likely to contain if we simply sequenced part of their mtDNA instead of testing for differences in restriction sites by assaying restriction enzymes at random. Furthermore, because we used heterologous primers designed for different families of insects (Bogdanowicz et al. 1993, Simon et al. 1994, Sperling & Hickey 1994), we wanted to provide the option of eventually designing primers that are an exact match to the *Yponomeuta* sequences. In addition, we wanted to lay a foundation that would allow a diagnostic method to be expanded to include new species without having to survey for new restriction sites in old species. Finally, because we were examining a region of mtDNA that had already been sequenced in a number of other families of Lepidoptera (e.g., Liu & Beckenbach 1992, Bogdanowicz et al. 1993, Brower 1994, Brown et al. 1994, Sperling & Hickey 1994), we wanted to contribute sequence data that would potentially be useful in higher-level phylogenetic comparisons.

The adult moths used for sequencing the 2.3-kb region were frozen live in 1993 and stored at −70°C for several months before extracting their DNA. The three specimens included one *Y. padella* from Victoria, British Columbia, ex *Crataegus monogyna* Jacq.; one *Y. malinellus* from Victoria, British Columbia, ex *Malus* sp.; and one *Y. cagnagella* from Ottawa, Ontario, ex. *Euonymus sanguinea* + *alata* × *europaea.* Methods for DNA extraction, amplification using the polymerase chain

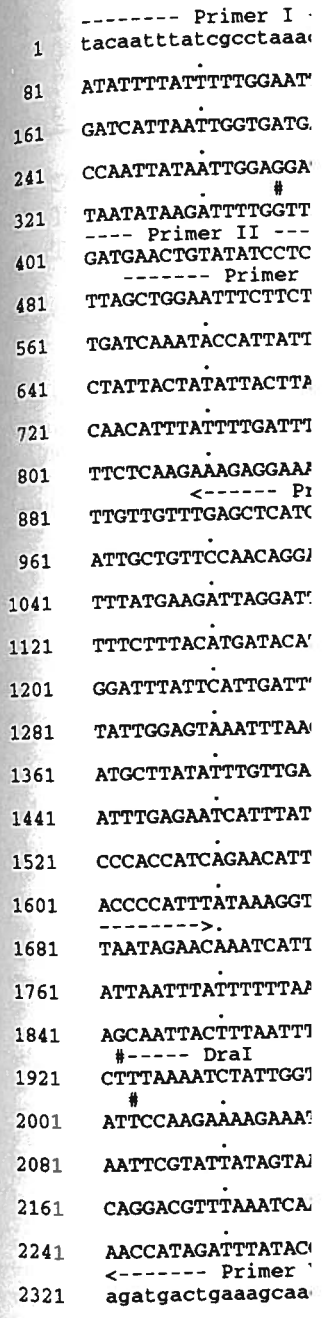

Fig. 1. DNA sequences for (lower case) are included to m different in *Y. padella* or *Y. ca* dashes.

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used for sequencing the 2.3-kb
n live in 1993 and stored at
months before extracting their
specimens included one *Y. pa-*
, British Columbia, ex *Crataegus*
ne *Y. malinellus* from Victoria,
ex *Malus* sp.; and one *Y. cag-*
wa, Ontario, ex. *Euonymus san-*
europaea. Methods for DNA ex-
tion using the polymerase chain


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-------- Primer I ------->
1    tacaatttatcgcctaaacttcagccATTTTATTAGCGAAAATGATTATATTCAACAAATCATAAAGATATTGGAACATT
81   ATATTTTATTTTTGGAATTTGATCTGGAATAGTAGGTACATCCCTAAGATTATTAATTCGGGCTGAATTAGGAAATCCTG
161  GATCATTAATTGGTGATGATCAAATTTATAATACTATTGTTACAGCTCATGCTTTTATTATAATTTTTTTTATAGTTATA
                              -#---- SpeI
241  CCAATTATAATTGGAGGATTTGGAAATTGATTAGTCCCTTTAATATTAGGAGCTCCTGATATAGCTTTCCCACGTATAAA
321  TAATATAAGATTTTGGTTATTACCTCCATCCTTAACACTATTAATTTCTAGAAGAATTGTAGAAAACGGAGCTGGAACAG
     ---- Primer II -----#
401  GATGAACTGTATATCCTCCTCTATCATCTAATATCGCACACAGAGGAAGATCTGTAGATTTAGCAATTTTCTCATTACAT
       ------- Primer III ------>.
481  TTAGCTGGAATTTCTTCTATTTTAGGAGCAATTAATTTTATTACAACTATTATTAATATAAAAAGTAATGGTATATCATT
561  TGATCAAATACCATTATTTGTTTGAGCTGTAGGAATTACAGCATTATTATTATTATTATCTTTACCTGTACTAGCTGGAG
                                                          #----- BamHI   #
641  CTATTACTATATTACTTACAGATCGAAATTTAAATACTTCTTTTTTTGACCCTGCTGGAGGGGGAGATCCTATTTTATAT
                  -#---- BanI
721  CAACATTTATTTTGATTTTTTGGACACCCAGAAGTATATATTTTAATTTTACCTGGATTTGGAATAATTTCACATATTAT
801  TTCTCAAGAAAGAGGAAAAAAAGAAACATTTGGAAATTTAGGAATAATCTATGCCATAATAGCAATTGGTTTACTTGGAT
       <------ Primer IV -----
881  TTGTTGTTTGAGCTCATCATATATTTACTGTTGGTATAGATATTGATACACGAGCTTATTTTACATCTGCAACTATAATT
961  ATTGCTGTTCCAACAGGAATTAAAATTTTTAGTTGATTAGCAACTTTACATGGAACTCAAATTAATTATAGTCCTTCAAT
1041 TTTATGAAGATTAGGATTTGTATTTTTATTTACAGTAGGAGGATTAACAGGAGTAATCTTAGCTAATTCTTCTATTGATG
1121 TTTCTTTACATGATACATATTATGTAGTAGCTCATTTTCATTATGTTTTATCTATAGGAGCTGTATTTGCTATTATAGCA
1201 GGATTTATTCATTGATTTCCTTTATTTACAGGACTAACATTAAACCCTTATTTATTAAAAATTCAATTTTTAACTATATT
1281 TATTGGAGTAAATTTAACATTTTTTCCTCAACACTTTTTAGGGTTAGCTGGTATACCTCGACGTTACTCTGATTATCCTG
1361 ATGCTTATATTTGTTGAAATATTATTTCTTCTTTAGGATCCTATATTTCATTTTTAGCAATTATAATAATATTAATTATT
1441 ATTTGAGAATCATTTATTTCTCAACGAATAATTTTATTTTCATTAAACATACCTTCTTCTATTGAATGACTCCAAAAATT
                                                  --------- Primer V ------->
1521 CCCACCATCAGAACATTCATATAATGAACTTCCTATTTTAAGAAACTTCTAATATGGCAGACTATATGTAATGGATTTAA
                                                                  --- Primer VI
1601 ACCCCATTTATAAAGGTTAATCCTTTTTTTAGAAATGGCAACATGAAATAATTTAAATTTACAAAATGGAGCATCTCCTT
-------->.          #----- BclI
1681 TAATAGAACAAATCATTTTTTTTCACGATCATACATTAATTATTTTAATTATAATTACAATCTTAGTAGGATATTTAATA
1761 ATTAATTTATTTTTTAATAAATATATTAATCGATTTTTATTAGAAGGACAAATAATTGAATTAATTTGAACAATTTTACC
1841 AGCAATTACTTTAATTTTTATTGCTCTTCCATCTCTTCGTTTATTATATTTATTAGATGAACTTAATAATCCTTTAATTA
  #----- DraI      .<---- Primer VII ---
1921 CTTTAAAATCTATTGGTCATCAATGATATTGAAGTTATGAATATTCAGATTTTAATAATATTCAATTTGATTCTTATATA
  #                                                                  #.
2001 ATTCCAAGAAAAGAAATAAAATTTAATGAATTTCGATTATTAGATGTAGATAATCGTATTATTCTTCCCATAAATAACCA
2081 AATTCGTATTATAGTAACAGCAACAGATGTAATTCATTCTTGAACAGTTCCATCTTTAGGAGTTAAAATTGATGCTAATC
2161 CAGGACGTTTAAATCAAACAAATTTTTTCATTAATCGTCCTGGATTATTTTATGGACAATGTTCTGAAATTTGTGGAGCA
2241 AACCATAGATTTATACCTATTGTAATTGAAAGAATTTCAATTAATAATTTTATTAAATGAATTAATAATTACTCTTCATT
     <------- Primer VIII ------
2321 agatgactgaaagcaagtaatggtctc
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Fig. 1. DNA sequences for *Y. malinellus* across mitochondrial COI, COII, and tRNA leucine genes. End primers (lower case) are included to make fragment sizes consistent with Fig. 3. Number signs (#) are above bases that are different in *Y. padella* or *Y. cagnagella*. Diagnosically informative restriction site primer locations are indicated by dashes.

reaction (PCR), and sequencing follow Sperling et al. (1994). Mitochondrial DNA was sequenced over a 2,294 base pair (bp) region that begins in the gene for tRNA tyrosine, extends through the genes for cytochrome oxidase subunits 1 and 2 and the tRNA leucine gene, and ends in the tRNA lysine gene. This region is homologous to bases 1,461–3,771 in *Drosophila yakuba* Burla (Clary & Wolstenholme 1985). Overlapping sections of this region were PCR-amplified using heterologous primers primarily developed for use with the spruce budworm (Sperling & Hickey 1994). Most of these primers had one mismatch to the *Yponomeuta* sequence. The fragments were then se-

	1	2	3	3	3	4	4	5	7	7	7	1	1	1	1	1	1	1	2	2	2
	7	4	7	0	3	8	2	4	8	0	2	4	9	5	4	2	5	0	2	0	7	8
	5	1	1	9	6	7	1	1	2	5	0	4	2	8	5	3	4	6	2	3	9	5
Y. malinellus	A	G	T	C	G	C	C	C	T	A	T	A	A	T	C	G	T	C	T	T	C	T
Y. padella	G	A	C	T	A	T	T	T	G	A	C	G	T	C	T	A	C	T	A	C	T	C
Y. cagnagella	G	A	T	T	G	T	T	T	G	G	C	G	T	C	T	A	T	T	A	C	T	C

Fig. 2. Mitochondrial DNA sites that vary among three *Yponomeuta* specimens sequenced over a 2,294-bp region. Numbering of sites corresponds to Fig. 1.

quenced directly using ABI automated sequencing with fluorescent dye terminators (Applied Biosystems, Foster City, CA). The three sequences were searched for potential diagnostic restriction site differences using 129 recognition sequences in the MicroGenie sequence analysis program (Beckman Instruments, Palo Alto, CA).

Subsequent analyses were done using specimens from 41 additional collections, most of which were air dried. These were museum specimens collected five or less years ago, except for one specimen collected in 1972. In total, 25 collections of *Y. malinellus*, 14 of *Y. padella*, and 5 of *Y. cagnagella* were sampled. Only adults reared from wild-collected larvae were used, and identifications were based on the larval host. Because ermine moth females lay their eggs in batches and, therefore, collections from a single plant are likely to be siblings, only a single specimen is reported for each locality, host, or period. However, two to three additional siblings were assayed for some batches to confirm unusual genotypes and lack of variation within batches. Only the head, thorax, and legs of each specimen were used for DNA extraction. The remaining body parts, including wings and abdomen, were placed in a gelatin capsule and deposited in the Canadian National Collection as vouchers.

The DNA from dried specimens was extracted using the protocols of Bogdanowicz et al. (1993) or Saghai-maroof et al. (1984), but without using proteinase K. Both extraction methods produced DNA that was sufficiently intact to consistently allow amplification of three different fragments of ≈400-bp length. Four specimens could not be amplified over one region using a primer pair that would have produced a fragment of 392 bp, but were successfully amplified using a primer pair giving a fragment of 293 bp. The three separate fragments used for diagnostics each contained two variable restriction sites. All three fragments were amplified separately from each specimen, which gave an internal control for sample identity and restriction site variants. Restriction site digestions were performed as per manufacturer specifications (New England Biolabs, Beverly, MA), and fragments were visualized with ethidium bromide staining of 1.5% agarose gels. To find further variation within the *Y. padella* complex, two additional specimens of each species were sequenced over the first 373 bp of the COI gene. One *Y. multipunctella* was also sequenced over this region and characterized with restriction enzymes over the same three regions as the other species. This *Y. multipunctella* specimen was collected in Cass County, Michigan.

Results

Sequence Variation. The full sequence of 2,294 bp is reported here for *Y. malinellus* (Fig. 1), and has been deposited in GENBANK under accession no. UO9206. In total, 22 sites differed among the sequences of *Y. malinellus*, *Y. padella*, or *Y. cagnagella* (Fig. 2). The most similar pair was *Y. padella* and *Y. cagnagella*, which had only four nucleotide differences (0. 2% divergence). *Y. padella* and *Y. cagnagella* had 21 and 19 differences from *Y. malinellus*, respectively (0.9 and 0.8% divergence). Only 3 of the 22 variable sites showed transversions, and the sequences had an A+T proportion of 74.5%. All substitutions were synonymous and 20 were at third position sites whereas two were at the first position in a leucine codon. There were no insertions or deletions. Thus the pattern of sequence divergence was consistent with that observed for the earliest stages of divergence in the mtDNA of *Drosophila* and spruce budworm moths (Beckenbach et al. 1993, Sperling & Hickey 1994).

No new genotypes were found in the six additional specimens of the *Y. padella* group that were sequenced over 373 bp. In contrast, preliminary sequence analysis showed that *Y. multipunctella* had >6% divergence from the *Y. padella* complex over this region. We did not sequence *Y. multipunctella* further, because restriction site differences were already apparent with the enzymes that were used to distinguish among members of the *Y. padella* complex.

Restriction Site Survey. Genotypic differences among collections of the *Y. padella* complex were assayed using six restriction sites in three fragments (Fig. 3). Four of these sites distinguished *Y. malinellus* from both *Y. padella* and *Y. cagnagella*, whereas one (Spe I in fragment 1) distinguished some specimens of *Y. padella* from the other two

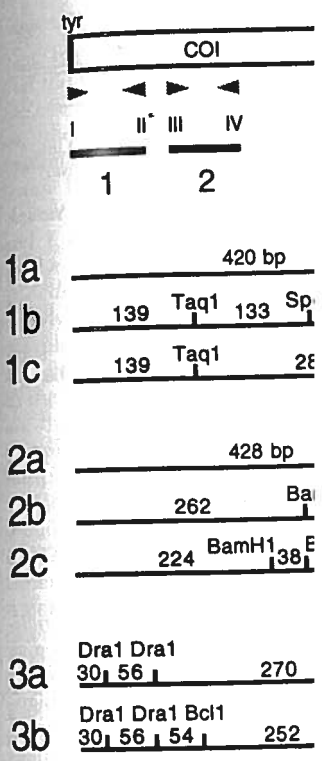

Fig. 3. Location of amplified striction sites, and primers used the *Y. padella* complex. Primer low and are followed by their l of *Drosophila yakuba* (Clary a Primer VI is included as an alt TACAATTTATCGCCTAAACT −s); II, 5′ AGGAGGATAAACA 1840 −a); III, 5′ GCTGGAA GAGC 3′ (3′@ 1949 −s); IV, 5′ GAGCTCA 3′ (3′@ 2329 −a); GATTATATGTAATGGA 3′ (AGAGCCTCTCCTTTAATAGA VII, 5′ TCATAAGTTCAA/GT −a); VIII, 5′ GAGACCATTAA 3′ (3′@ 3771 −a).

species, and one (BamHI guished *Y. cagnagella* from *Y. malinellus*. In combinatic five genotypes in the *Y. pad* notype was found in *Y. mu* trated), which had only the

1	1	1	1	2	2	2
3	5	7	9	0	0	2
2	5	0	2	0	7	8
3	4	6	2	3	9	5
G	T	C	T	T	C	T
A	C	T	A	C	T	C
A	T	T	A	C	T	C

quenced over a 2,294-bp region.

ecies were sequenced over e COI gene. One *Y. multi-* uenced over this region and striction enzymes over the s the other species. This *Y.* nen was collected in Cass

Results

n. The full sequence of 2,294 or *Y. malinellus* (Fig. 1), and GENBANK under accession , 22 sites differed among the *inellus*, *Y. padella*, or *Y. cag-* most similar pair was *Y. pa-lla*, which had only four nu- (0. 2% divergence). *Y. padella* d 21 and 19 differences from ctively (0.9 and 0.8% diver- the 22 variable sites showed e sequences had an A+T pro- All substitutions were synony- at third position sites whereas st position in a leucine codon. ertions or deletions. Thus the ce divergence was consistent for the earliest stages of diver- NA of *Drosophila* and spruce eckenbach et al. 1993, Sperling

es were found in the six addi- f the *Y. padella* group that were 73 bp. In contrast, preliminary showed that *Y. multipunctella* ce from the *Y. padella* complex We did not sequence *Y. multi-* because restriction site differ- apparent with the enzymes that nguish among members of the *Y.*

e Survey. Genotypic differences of the *Y. padella* complex were restriction sites in three frag- our of these sites distinguished *Y.* oth *Y. padella* and *Y. cagnagella*, e I in fragment 1) distinguished of *Y. padella* from the other two

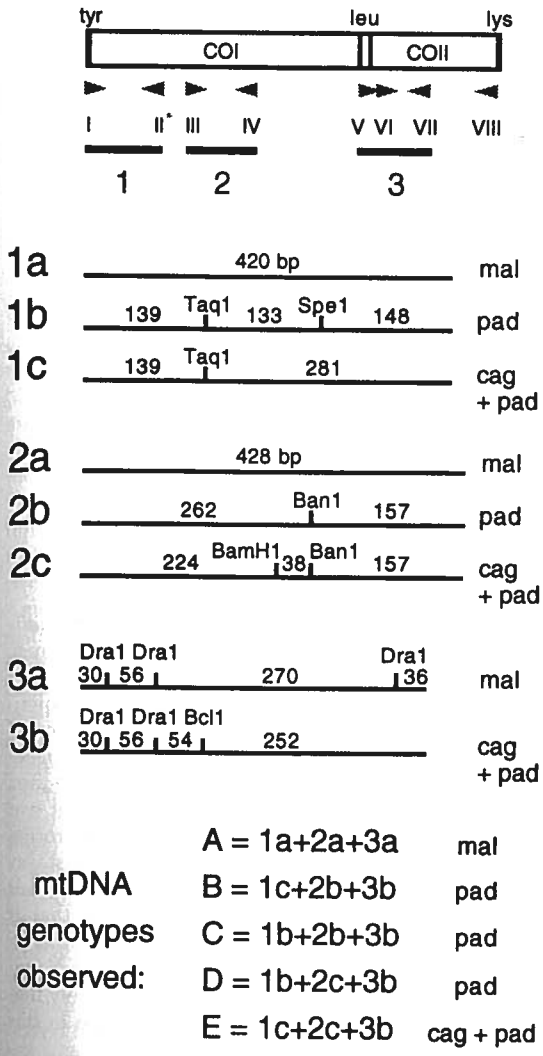

Fig. 3. Location of amplified fragments, diagnostic restriction sites, and primers used to assay genotypes within the *Y. padella* complex. Primer sequences are given below and are followed by their locations in the sequence of *Drosophila yakuba* (Clary and Wolstenholm 1985). Primer VI is included as an alternate to primer V. I, 5′ TACAATTTATCGCCTAAACTTCAGCC 3′(3′@ 1460 −s); II, 5′ AGGAGGATAAACAGTTCA(C/T)CC 3′ (3′@ 1840 −a); III, 5′ GCTGGAATTTCTTCTATTTTAGGAGC 3′ (3′@ 1949 −s); IV, 5′ ACTGTAAATATATGATGAGCTCA 3′ (3′@ 2329 −a); V, 5′ CTAATATGGCAGATTATATGTAATGGA 3′ (3′@ 3038 −s); VI, 5′ AGAGCCTCTCCTTTAATAGAACA 3′ (3′@ 3138 −s); VII, 5′ TCATAAGTTCAA/GTATCATTG 3′ (3′@ 3389 −a); VIII, 5′ GAGACCATTAATTGCTTTCAGTCATCT 3′ (3′@ 3771 −a).

species, and one (BamHI in fragment 2) distinguished *Y. cagnagella* from some *Y. padella* and all *Y. malinellus*. In combination, these six sites gave five genotypes in the *Y. padella* group. A sixth genotype was found in *Y. multipunctella* (not illustrated), which had only the Bcl I site of the six diagnostic sites in the *Y. padella* group. It also had a new TaqI site that cut fragment 1 into two pieces of 52 and 367 bp.

Four restriction site genotypes were found in material assayed from North America (Table 1); the fifth genotype (B) was found in European material. The 25 collections of *Y. malinellus* all had the A genotype. The five *Y. cagnagella* assayed all had the E genotype, including one European and four North American specimens. In contrast, all five genotypes were found in specimens identified as *Y. padella* on the basis of host affiliation.

Most collections of *Y. padella* had a C genotype, but two had an E genotype as in *Y. cagnagella*. One of these two was also sequenced over fragment 1 and showed the same sequence as *Y. cagnagella*. The identity of the two collections of *Y. padella* that had the E genotype is supported by wing coloration characteristics. Several specimens in each of these two collections had a smoky suffusion extending to the base of the wings, a characteristic that is found only in *Y. padella*. The D genotype was found in two collections of *Y. padella*, including one from the Bellingham area of Washington (collected by Levi Strauss, Washington State Department of Agriculture). The Washington sample represents the first confirmed *Y. padella* from the western United States.

One collection from Boundary Bay, BC, was labeled as *Y. padella* from hawthorne, but had the A genotype that was otherwise characteristic of *Y. malinellus*. This was confirmed by assaying a total of three specimens (presumably siblings) from the same collection. As a further check, we dissected the genitalia of eight specimens from Boundary Bay collections (one male and one female reared from *Malus* sp. and two males and four females reared from *Crataegus* sp.) and assigned identifications using discriminant functions and keys provided in Povel (1984). The results were inconclusive, although it should be noted that Povel (1984) found that only 58–92% of *Y. padella* and *Y. malinellus* were correctly identified with these discriminant functions, depending on sex and suite of characters considered. None of the specimens in this collection had the smoky wing suffusion that clearly identifies some specimens of *Y. padella*.

Discussion

Dried adult specimens of the *Y. padella* complex are currently very difficult to identify without host data (Menken et al. 1993), and yet the distinct nature of these species must be taken into account in any effort to control them. Of the three species introduced to North America, *Y. cagnagella* probably represents the most benign problem, because it is only an occasional pest on ornamental shrubs (Hoebeke 1987). However, *Y. malinellus* has already reached damaging numbers in apple orchards in Washington and British Columbia (Unruh et al. 1993), and *Y. padella* has caused

Table 1. Mitochondrial genotypes and larval hosts of reared samples, grouped by hosts characteristic for each *Yponomeuta* species

Larval Host	Genotypes, no. independent collections, and locations
	Y. malinellus (n = 25)
Malus pumila Mill.	A × 14 (British Columbia: Barrier, Cache Creek, Comox, D'Arcy, Kamloops, Little Fort, Lund, Lytton, Malakwa, Saanichton, Sardis, Sechelt, Spuzzum, Vancouver)
Malus diversifolia (Bong.) Roem	A × 2 (British Columbia: Shawnigan Lake, Sidney)
Malus sp.	A × 9 (British Columbia: Boundary Bay, Duncan, Ganges, Ladysmith, Sidney, Victoria; Ontario: Whilby; Nova Scotia: Kentville; Washington: Bellingham)
	Y. padella (n = 14)
Amelanchier alnifolia Nutt.	C × 1 (British Columbia: Sidney)
Crataegus monogyna Jacq.	B × 2 (Netherlands: Leiden, Bierlap), C × 1 (British Columbia: Victoria), D × 1 (Washington: Bellingham), E × 1 (British Columbia: Ladner)
Crataegus laevigata (Poir.)	C × 1 (British Columbia: Victoria)
Crataegus sp.	A × 1 (British Columbia: Boundary Bay), D × 1 (British Columbia: Boundary Bay)
Prunus cerasifera J.F.Ehrh.	C × 2 (British Columbia: both at Victoria)
Prunus insititia L.	C × 1 (British Columbia: Victoria)
Prunus sp.	E × 1 (British Columbia: Boundary Bay)
Sorbus aucuparia L.	C × 1 (British Columbia: Victoria)
	Y. cagnagella (n = 5)
Euonymus europaea L.	E × 2 (Ontario: Ottawa; Massachusetts: Lincoln)
Euonymus sp.	E × 3 (Ontario: Ottawa; Michigan: East Lansing; Austria: Vienna)

economic losses on cherry and plum trees in Europe (Menken et al. 1993). The collection of hawthorne-feeding *Yponomeuta* from Washington now confirms the presence of *Y. padella* in the western United States as well as in Canada, and emphasizes the need for additional means to distinguish *Y. padella* from *Y. malinellus*.

Diagnostic identification based on mtDNA appears to effectively distinguish *Y. malinellus* and *Y. padella* in North America (Fig. 4). In fact, three separate, informative fragments can readily be amplified from DNA extracted from museum specimens. This diagnostic method can easily be improved by designing primers that give a perfect match with the *Yponomeuta* sequence (Fig. 1) and that are also better located to give distinct fragment patterns. Although one collection of hawthorne-feeding *Yponomeuta* from Boundary Bay had the A genotype, which characterizes *Y. malinellus* (Table 1), we suggest that this collection may have been mislabeled. Identification of this material using morphological characters was ambiguous, a situation that is common for collections of these two species (Povel 1987). A follow-up collection of larvae from hawthorne from the same area in 1994 gave specimens with a genotype that was more typical of *Y. padella*. Continued monitoring of the Boundary Bay area remains the simplest way to determine whether rare *Y. padella* individuals may indeed contain a mtDNA genotype that is more typical of *Y. malinellus*. Because *Y. malinellus* has been shown to accept no host plants of *Y. padella* (Kooi 1988), we do not consider it likely that *Y. malinellus* simply has a wide host range in this area.

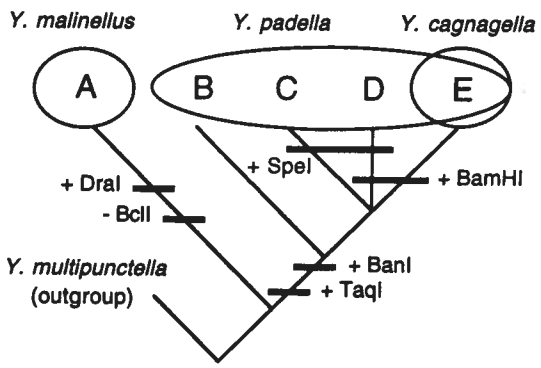

Fig. 4. Dendrogram depicting genetic relationships among mtDNA genotypes found in *Yponomeuta*. Restriction site gains (+) or losses (-) are optimized using *Y. multipunctella*. Of the six sites surveyed in the *Y. padella* complex, *Y. multipunctella* had only the BclI site and a new TaqI site at bp 367.

Yponomeuta padella has only been recorded in British Columbia since 1992 (Wood & Van Sickle 1994). However, at least three mtDNA genotypes are found in these populations. In fact, the genotypes occur in different areas. All collections made near Victoria (Victoria and Sidney) had the C genotype, whereas the collections made near Vancouver, BC, (Ladner and Boundary Bay) and in Washington had the D or E genotype. Thus, the progeny of at least three different females appear to have been introduced into British Columbia or Washington in recent years, and we suggest that separate introductions may have been made to two different areas.

Although most of the specimens of *Y. padella* and *Y. cagnagella* had different mtDNA genotypes, a small number of *Y. padella* had mtDNA that was not distinguished from that of *Y. cagnagella* (Table 1; Fig. 4). The two European specimens of *Y. padella* support the close similarity of the species, because they had a genotype that may have branched off before the divergence of the geno-

types that are found in North
clear from their low mtDNA se
that the two species are very clo
theless, it is possible that other
or other methods, such as sin
mational polymorphism analysi
baum 1993), will reveal substit
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and *Y. cagnagella*.

The close relationship betwe
Y. padella and *Y. cagnagella* do
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and allozymes, as well as host a
to the conclusion that *Y. padell*
are the most closely related pa
complex (Menken et al. 1993).
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species are very closely related
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mtDNA clearly shows the clos
tween *Y. padella* and *Y. cagna*
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Yponomeuta species may legiti
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Although the mtDNAs of
padella + *Y. cagnagella* were
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Sequence divergences of 1–
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culifera (Guenée) (Sperlin
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C. occidentalis (Sperling and
fore, we believe it will be par
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complex in Europe before an
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Such a survey could potentiall
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Ganges, Ladysmith, Sidney, Victoria; ton: Bellingham)

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British Columbia: Boundary Bay)

Austria: Vienna)

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types that are found in North America. It is also clear from their low mtDNA sequence divergence that the two species are very closely related. Nonetheless, it is possible that other regions of mtDNA or other methods, such as single-strand conformational polymorphism analysis (Lessa & Applebaum 1993), will reveal substitutions that allow a clearer distinction to be made between *Y. padella* and *Y. cagnagella*.

The close relationship between the mtDNA of *Y. padella* and *Y. cagnagella* does not support earlier hypotheses of relationship within the species complex. Extensive examination of morphology and allozymes, as well as host associations, had led to the conclusion that *Y. padella* and *Y. malinellus* are the most closely related pair of species in the complex (Menken et al. 1993). Nonetheless, both morphology and allozymes indicate that all three species are very closely related (Menken 1982, Arduino & Bullini 1985, Povel 1987). In contrast, mtDNA clearly shows the closest relationship between *Y. padella* and *Y. cagnagella* among North American *Yponomeuta*. One explanation for this discrepancy is that the mtDNA phylogeny of these *Yponomeuta* species may legitimately be different from their species phylogeny (=average gene phylogeny). Incongruent phylogenies are plausible because mtDNA is only a single linked locus, and therefore gene introgression and retained ancestral polymorphism can cause it to have a different phylogeny from the average gene phylogeny (Avise 1991).

Although the mtDNAs of *Y. malinellus* and *Y. padella* + *Y. cagnagella* were distinct, with 0.8–0.9% sequence divergence between them, this degree of divergence is relatively small in comparison to that found within other species of Lepidoptera. Sequence divergences of 1–2% have been observed for the COI-COII region of mtDNA within the western spruce budworm, *Choristoneura occidentalis* Freeman; hemlock looper, *Lambdina fiscellaria* (Guenée); and dingy cutworm, *Feltia jaculifera* (Guenée) (Sperling & Hickey 1994; unpublished data), and divergences of >2% have been estimated for races of the gypsy moth, *Lymantria dispar* (L.) (Bogdanowicz et al. 1993). However, *Choristoneura pinus* Freeman shows only 0.6–0.8% divergence from its sister species, *C. occidentalis* (Sperling and Hickey 1994). Therefore, we believe it will be particularly important to survey the mtDNA of the species of the *Y. padella* complex in Europe before any further conclusions can be drawn regarding their mtDNA phylogeny. Such a survey could potentially give a more precise location for the source of introductions of these species from Europe and will certainly contribute to a better understanding of the mode of speciation of a well-studied group of model insects. Meanwhile, the ability to differentiate dried specimens of *Y. malinellus* from *Y. padella* in British Columbia, Washington, and Oregon should allow the expanding distributions of these species to be mapped more precisely and control measures to be focused more effectively.

Acknowledgments

We thank R. Duncan (Canadian Forest Insect and Disease Survey), B. Gill, and D. Parker (Agriculture Canada); S. Passoa (USDA–APHIS); and Levi Strauss and Eric LaGasa (Washington State Department of Agriculture) for providing ermine moth specimens used in this study. Primers VII and VIII were kindly donated by the laboratory of R. Harrison (Cornell University). Constructive criticism was provided by two anonymous reviewers. We thank R. Barrette and R. Foottit (Agriculture Canada) for making laboratory facilities available for supplementary testing of the mtDNA diagnostic method. Funding was provided by a National Science and Engineering Research Council of Canada operating grant to D.A.H., a postdoctoral fellowship from Insect Biotech Canada to F. Sperling, and The Centre for Land and Biological Resources Research (Agriculture Canada) funding to J.-F.L.

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Received for publication 17 May 1994; accepted 28 October 1994.

Entrapment of

Department of

ABSTRACT Aphids often rupture latex car become trapped in ex propensity for trappin duction of aphids anc black seeded Simpsor ville, MD, were *Urole* 79% of the *U. pseuda* glued to the lettuce p walking across the pl lettuce bracts and bu on stalks and leaves v and buds, the females died as a result. Over only 2% of the free whiteflies, *Bemisia ta* trapped in latex, prim document the potenc sives in discouraging

KEY WORDS *Uro*

MANY PLANTS SEQUESTER vis as latex or resin, within spec distinct from the vascular tiss calfe & Chalk 1983, Farrell e often ramify throughout the p orate network. Secretions ar der pressure (Buttery & Boat al. 1989). Thus, when a pla exudate flows from the plan of damage, immediately up tions frequently contain toxir also often coagulate upon threatening prospective herb entrapment in hardening ex ander 1982, Farrell et al. 19

Canal-borne secretions cl fect on mandibulate insect dates often deter feeding, insects outright by sealing t the insect to the plant, or by insect in hardening secretio 1982, Farrell et al. 1991, Za Dussourd & Denno 1994) have been observed immol cluding caterpillars (Benr Brower 1992, Dussourd

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