Available online at www.sciencedirect.com
SCIENCE DIRECT®
Molecular Phylogenetics and Evolution 32 (2004) 207–220
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Molecular phylogeny of *Cotesia* Cameron, 1891 (Insecta: Hymenoptera: Braconidae: Microgastrinae) parasitoids associated with Melitaeini butterflies (Insecta: Lepidoptera: Nymphalidae: Melitaeini)

Maaria Kankare[a,*] and Mark R. Shaw[b]

[a] *Department of Biological and Environmental Sciences, Division of Population Biology, University of Helsinki, P.O. Box 65, FIN-00014, Finland*
[b] *National Museums of Scotland, Chambers Street, Edinburgh, EH1 1JF, UK*

Received 10 July 2003; revised 6 November 2003
Available online 31 January 2004

Abstract

Phylogenetic relationships among *Cotesia* Cameron (Braconidae) species parasitising Melitaeini butterflies were examined using DNA sequence data (mitochondrial cytochrome oxidase subunit I and NADH1 dehydrogenase genes, nuclear ribosomal DNA internal transcribed spacer region) as well as 12 microsatellite loci. Molecular data were available from ostensibly six species of *Cotesia* from 16 host butterfly species in Europe, Asia, and North America. Analysis of the combined sequence data using both maximum parsimony and maximum likelihood revealed two distinct *Cotesia* clades. In one clade (*C. acuminata* (Reinhard); *C. bignellii* (Marshall)) host ranges are apparently narrow and, although *Euphydryas* (s. lato) is well-utilised, permeation of *Melitaea* (s. lato) has been slight. In the other clade (*C. melitaearum* (Wilkinson); *C. lycophron* (Nixon); *C. cynthiae* (Nixon)) host utilization across the Melitaeini as a whole is more extensive and the data are consistent with more recent, or active, speciation processes. Neighbour-joining trees calculated separately for the two main clades based on Cavalli-Sforza and Edwards (1967) chord distance (D_{CE}) of microsatellite allele frequencies were consistent with phylogenetic trees obtained from the sequence data. Our analysis strongly suggests the presence of several additional, previously unrecognised, *Cotesia* species parasitising this group of butterflies.

Keywords: *Cotesia*; Phylogeny; Parasitoids; Melitaeini; mtDNA; Microsatellites

1. Introduction

Cotesia Cameron (Hymenoptera: Braconidae: Microgastrinae) is a large genus of primary parasitoids, with an estimated world fauna of ca. 1500–2000 species (Mason, 1981). It is entirely associated with lepidopteran hosts (Shaw and Huddleston, 1991), records from other insects almost certainly being erroneous. Many *Cotesia* species are important natural enemies of agricultural and forestry pests, and a few have been manipulated as biocontrol agents. One, *C. glomerata* (Linnaeus), is a common parasitoid of the Eurasian cabbage white butterflies (species of *Pieris* Schrank) and has been studied in considerable detail both in the laboratory and in the field, with the generation of a vast associated literature. The current usage of the generic name *Cotesia* is relatively recent (Mason, 1981), and the previous literature pertaining to *Cotesia* species used the traditional name *Apanteles* Foerster (which now has a more restricted application: cf. Mason, 1981).

Several species of *Cotesia* are key parasitoids of Melitaeini butterflies in Eurasia and North America (Erlich and Hanski, 2004). Melitaeini is a distinct group in the traditional family Nymphalidae, consisting of ca. 250 species widely distributed in Europe, Asia, and North and South America (Higgins, 1981; Wahlberg and Zimmermann, 2000). Owing to their population structures Melitaeini have been widely used in ecological and evolutionary studies for more than four decades

* Corresponding author. Fax: +358-9-191-57694.
E-mail address: maaria.kankare@helsinki.fi (M. Kankare).

1055-7903/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2003.11.013

(e.g. Erlich et al., 1975; Erlich and Hanski, 2004; Hanski, 1999; Thomas and Singer, 1998).

Cotesia species are all koinobiont endoparasitoids—that is, their host continues to develop after the female parasitoid has oviposited into it. Koinobiont parasitic Hymenoptera necessarily have an intricate physiological relationship with their host and consequently tend to have relatively narrow host ranges (Askew and Shaw, 1986; Haeselbarth, 1979). The *Cotesia* species parasitising Melitaeini are not known to parasitize any other species of butterflies (despite some records in the literature which should be discounted for the reasons given by Shaw, 1994). As they are specialist parasitoids, the population dynamics of these *Cotesia* species can be strongly coupled with the population dynamics of their hosts (Lei and Hanski, 1997; Porter, 1981; van Nouhuys and Hanski, 2004). Because they can develop successive broods on a single host generation, and are gregarious parasitoids developing large broods when parasitising older hosts, the potential impact of these parasitoids on their host population is unusually large. The population ecology and biology of one species, *Cotesia melitaearum* (Wilkinson), has been intensively studied for 10 years in the Åland Islands in Finland (Lei et al., 1997; van Nouhuys and Hanski, 2004). Among other things, it has been shown that parasitism by *C. melitaearum* increases the risk of extinction of local populations of *Melitaea cinxia* (Linnaeus) (Lei and Hanski, 1997).

Altogether seven described species of *Cotesia* are known to parasitize melitaeine butterflies: *Cotesia acuminata* (Reinhard), *C. bignellii* (Marshall), *C. cynthiae* (Nixon), *C. lycophron* (Nixon), *C. melitaearum*, *C. euphydryidis* (Muesebeck), and *C. koebelei* (Riley). The first five species occur in Europe and Asia, while the last two species occur in North America. To judge from literature records and the sporadic presence of reared specimens in museum collections, some of these notional *Cotesia* species have appeared to be highly specialised, recorded from only a single host species throughout their range, while others have appeared to be capable of using many different melitaeine hosts. For the present work considerable effort was made to obtain fresh samples of *Cotesia* reared from as wide a range of Eurasian Melitaeini as possible, first in order to better understand the host relations of the notional (morpho-) species, second to analyze phylogenetic relationships both within and between these notional species using molecular methods, including testing the integrity of the notional species, and third to investigate the underlying evolutionary ecology and possible history of the host parasitoid relationships in so far as the molecular methodology, and host-range, morphological and natural history data, would allow. Although most effort was focused on the Eurasian taxa, the two North American species were included in our analyses to a limited extent.

It was not a purpose of the present work to investigate the internal phylogeny of *Cotesia* as a whole, and therefore we are unable to address the monophyly or otherwise of the *Cotesia* taxa that collectively attack Melitaeini. However, the traditional morphological treatment of European *Cotesia* (as the *Apanteles glomeratus*-group) by for example Nixon (1974) and Papp (1986, 1987) strongly suggests that two separate monophyletic clades are associated with Melitaeini. One (*C. acuminata* and *C. bignellii*) is morphologically isolated with respect to other *Cotesia*, its species sharing strong thoracic sculpture, smooth hind coxae, and a long acuminate hypopygium. The other (*C. melitaearum*, *C. cynthiae* and the enigmatic *C. lycophron*) is more similar to some other *Cotesia* species in the pattern of sculpture and the general form of the hypopygium, but the species attacking Melitaeini are nevertheless so close to one another that their separation can present problems, while being readily separable from other *Cotesia* through several characters. The original description of the taxon *C. lycophron* (Nixon, 1974) is of an extreme form (with a much reduced hypopygium), known to us only from the type series (a single reared brood) from a well-sampled host collected in a generally well-sampled part of France (Table 1). As we have occasionally seen similar reduction of the hypopygium in other *Cotesia* as an aberration, we believe that the type specimens of *C. lycophron* are aberrant and that ordinary specimens of the taxon to which it belongs are unlikely to have been distinguished from the notional species *C. melitaearum* sensu lato (Shaw, in prep).

In this paper we present a molecular phylogeny of *Cotesia* species associated with melitaeine butterflies, reared from a large number of populations of altogether 16 host species in Europe, Asia, and North America. Phylogenetic relationships among the *Cotesia* species were examined using DNA sequence data from mitochondrial cytochrome oxidase subunit I (COI) and NADH1 dehydrogenase (ND1) genes, from nuclear ribosomal DNA internal transcribed spacer region 2 (ITS2) and also from 12 microsatellite loci.

2. Materials and methods

2.1. Isolation and sequencing of DNA

Cotesia parasitoids were sampled fresh as cocoons from the field or (mostly) as reared adults from wild-collected hosts which were carefully identified. However, samples from some populations were only available as dried, pinned museum specimens. The Eurasian *Cotesia* samples were indentified by MRS and the North American samples by Kathleene Jensen, Karen Kester, Paul M. Marsh, and Jim B. Whitfield. Host identifications were made by the collectors. The number of *Cotesia* samples used in this study, with information on host

Table 1
Cotesia parasitising Melitaeini

Parasitoid	Host	Geographic area	Source**
Cotesia acuminata (Reinhard) agg.[a]	*Euphydryas maturna* (Linnaeus)	Europe	1, 2, 12,*
	Melitaea athalia (Rottemburg)	Europe	6,*
	Melitaea didyma (Esper)	Europe	7,*
	Melitaea latonigena Eversmann	Siberia	7,*
	Melitaea phoebe (Denis & Schiffermüller)	Europe, Siberia	6,*
	Melitaea scotosia Butler	China	15,*
Cotesia bignellii (Marshall)	*Euphydryas aurinia* (Rottemburg)	Europe	2, 5,*
Cotesia cynthiae (Nixon)	*Euphydryas cynthia* (Denis & Schiffermüller)	Europe	6,*
Cotesia euphydryidis (Muesebeck)	*Chlosyne harrisii* (Scudder)	North America-E	4
	Euphydryas phaeton (Drury)	North America-E	4
Cotesia koebelei (Riley)	*Chlosyne leanira* (C. & R. Felder)	North America	4
	Chlosyne neumoegeni (Skinner)	North America	4
	Euphydryas chalcedona (Doubleday & Hewitson)	North America-W	4
	Euphydryas editha (Boisduval)	North America-W	4, 9, 10
Cotesia lycophron (Nixon)	*Melitaea didyma* (Esper)	Europe	14
Cotesia melitaearum (Wilkinson) agg.[a]	*Euphydryas aurinia* (Rottemburg)	Europe	5, 7, 13,*
	Euphydryas aurinia davidi (Oberthür)	Siberia	7,*
	Euphydryas desfontainii (Godart)	Europe	1,*
	Euphydryas maturna (Linnaeus)	Europe	2,*
	Melitaea athalia (Rottemburg)	Europe	3, 7,*
	Melitaea cinxia (Linnaeus)	Europe, China	3,*
	Melitaea deione (Geyer)	Europe	*
	Melitaea diamina (Lang)	Europe	*
	Melitaea didyma (Esper)	Europe	*
	Melitaea parthenoides (Keferstein)	Europe	2, 8, 11,*
	Melitaea trivia (Denis & Schiffermüller)	Europe	12,*

Table modified from Table 8.2. (van Nouhuys and Hanski 2004).
[a] In the present work these traditional taxa are shown to be probably aggregates of closely related species.
* Voucher specimens in National Museums of Scotland.
** Source references: 1. Eliasson (1991); 2. Komonen (1997); 3. Lei et al. (1997); 4. Marsh (1979); 5. Porter (1981); 6. Williams et al. (1984); 7. Wahlberg et al. (2001); 8. S. van Nouhuys, pers. comm.; 9. White (1973); 10. Moore (1989); 11. Warren (1987); 12. Komonen (1998); 13. Ford and Ford (1930); 14. Nixon (1974); and 15. I. Hanski, pers. comm.

species, locations, and collectors, are detailed in Appendix A. DNA was extracted from a single individual or a cocoon using NucleoSpin Tissue Kit (Macherey-Nagel) according to the manufacturer's instructions except that 50 μl of milliQ water was used in the final elution stage.

DNA fragments from mtDNA COI and ND2 genes and from nuclear ITS2 region were used for the sequence analysis. Universal primers HCO1490 and LCO2198 (Folmer et al., 1994), and C1-J-1859, C1-J-2183, and TL2-N-3014 (Simon et al., 1994) were used to amplify part of the COI region, NDI F and R primers (Kambhampati and Smith, 1995) were used to amplify part of the NDI region and ITS2 F and R primers (van Veen et al., 2003) were used to amplify the ITS2 region. PCR consisted of 0.5 μM of each of the forward and reverse primers, 200 μM of each of the dNTPs, 1.5 mM $MgCl_2$, 20 ng of BSA and 0.5 U of Ampli *Taq* DNA polymerase (PE, Applied Biosystems). All amplifications were performed in 20 μl volumes using PTC 100 or PTC 200 thermal cyclers (MJ Research). PCR conditions were: denaturation at 95 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 49 °C for 1 min, and 72 °C for 1.5 min. Final extension was at 72 °C for 10 min. PCR products were purified using GFX purifying kit (Amersham–Pharmacia Biotech) and sequenced in both directions using BigDye terminator cycle sequencing kit (PE, Applied Biosystems) in 10 μl reaction volumes. Sequences were resolved on an ABI 377 automated DNA sequencer (PE, Applied Biosystems) and analyzed using ABI PRISM sequencing analysis software version 3.3 (PE, Applied Biosystems) with manual checking and aligning with SEQUENCHER version 3.0 (Gene Codes Corporation).

2.2. Microsatellite analysis

Twelve microsatellite loci were examined to help to elucidate the relationships of closely related species and populations (Takezaki and Nei, 1996). These 12 loci included in the analysis were: *Cme1*, *Cme3*, *Cme4*, *Cme15*, and *Cme17* isolated from *Cotesia melitaearum* (Kankare, Jensen, Kester, Saccheri, in prep.) and *Cco1A*, *Cco5A*, *Cco27*, *Cco42*, *Cco65A*, *Cco65B*, and *Cco68*, originally isolated from *Cotesia congregata* (Say) (Jensen et al., 2002). Twelve microsatellite loci were amplified in a total of eight PCRs, including two triplex and six single PCRs. One primer from each pair of primers was end-labelled with fluorescent dye (6-FAM, HEX, NED). Each single or multiplex PCR consisted of different concentrations of forward and reverse primers,

200 μM of each of the dNTPs, 1.5 mM $MgCl_2$, 20 ng of BSA and 0.5 U of Ampli *Taq* DNA polymerase. All amplifications were performed in 10 μl volumes using PTC 100 or PTC 200 thermal cyclers (MJ Research). PCR conditions were: denaturation at 94 °C for 2 min, followed by 35 cycles at 94 °C for 30 s, locus specific T_A for each PCR for 30 s and 72 °C for 45 s. Final extension was at 72 °C for 5 min. Diluted and pooled microsatellite PCR products were then resolved in three different panels in an ABI 377 automated DNA sequencer (PE, Applied Biosystems). Gels were analyzed and fragments sized using GENESCAN version 3.1.2 and GENOTYPER version 2.5 programs (PE, Applied Biosystems), respectively.

The Excel Microsatellite Toolkit (available at http://acer.gen.tcd.ie/~sdepark/ms-toolkit/) was used to estimate the genetic diversity in microsatellites. Nei's (1987) unbiased gene diversity (H_E), observed heterozygotes (H_o), mean number of alleles (A) and allele ranges for each *Cotesia* population over all loci are shown in Appendix B. Because of the haplodiploid nature of inheritance of *Cotesia*, only the data from females were used to calculate Nei's unbiased gene diversity and observed heterozygotes.

2.3. Phylogenetic analysis

2.3.1. DNA sequence analyses

Phylogenetic analyses were performed under the maximum parsimony (MP) and maximum likelihood (ML) criteria using PAUP version 4.0b10 (Swofford, 2002). A parasitoid wasp, *Microplitis xanthopus* (Ruthe), from another genus of the same subfamily (Microgastrinae) was included as outgroup in sequence analyses. Only one outgroup species was added to the analyses as our purpose was not to infer the monophyly or otherwise of the *Cotesia* parasitising Melitaeine butterflies but instead to study the relationships between these species. Sequences were aligned using program CLUSTAL W (Thompson et al., 1994) with the default alignment parameters. The sequences have been deposited in GenBank under Accession Nos. AY333869–AY333893 for COI, AY333894–AY333911 for ND1 and AY333847–AY333868 for ITS2.

Maximum parsimony analyses were conducted for the two mtDNA genes and the ITS2 region, both separately and combined, with the TBR branch swapping method, with 100 random additions of taxa and equal weight given to transitions and transversions. Gaps were treated as a fifth character. For the ML analysis, the most appropriate substitution model was selected with Hierarchical Likelihood Ratio Tests (hLRTs), implemented in MODELTEST program, version 3.06 PPC (Posada and Crandall, 1998). Bootstrap support values (Felsenstein, 1985) for each node in the MP and ML trees were calculated with 100 full heuristic searches, and 50% majority rule consensus trees were computed from these searches.

2.3.2. Microsatellites

Interspecific and intraspecific phylogenetic distances were estimated using stepwise mutation model (SMM; Ohta and Kimura, 1973) based $(\delta\mu)^2$ (Goldstein et al., 1995), and R_{ST} (Slatkin, 1995), and Cavalli-Sforza and Edwards (1967) chord distance (D_{CE}). Genetic distance estimate and boostrapping procedures were performed using the program MsatBoot version 1.2 (Landry et al., 2002). The resulting genetic distance matrices were used to construct Neighbor-Joining (N-J) and consensus trees with programs NEIGHBOR and CONSENSE, respectively, implemented in PHYLIP version 3.75c (Felsenstein, 1995). Treeview version 1.6.6 (R. D. M. Page 2001, available at: http://taxonomy.zoology.gla.ac.uk/rod/rod.html) was used to draw the trees. The chord distance (D_{CE}) of Cavalli-Sforza and Edwards (1967) has been suggested to be one of the best metrics to construct a reliable tree topology in closely related species (Takezaki and Nei, 1996). Because of this, the final microsatellite trees were constructed based on the chord distance (D_{CE}), separately for the two main clades produced by the analyses of the sequence data (Figs. 3 and 4). Since one Cco-locus (*Cco42*) and four Cme-loci (*Cme1, 3, 15, 17*) failed to amplify for almost all of the *C. acuminata* or *C. bignellii* individuals, these loci were removed from the analysis of clade A.

3. Results

3.1. Sequence data

Altogether about 2400 bp were sequenced from mtDNA COI and ND2 genes and from the nuclear region ITS2 for five notional species of *Cotesia*, reared from 16 host butterfly species from Europe, Asia, and North America. The alignments of the combined data set resulted in a total of 2426 nucleotide sites, of which 666 (27.5%) were variable and 367 (15.1%) were parsimony informative.

In the ML analysis, the most appropriate substitution model for COI was general time reversible (GTR; Yang, 1994) with modified proportion of invariable sites and gamma distribution (I + G). For ND1, the model selected was the transitional model (TIM; Rodreguez et al., 1990) with modified proportion of invariable sites and gamma distribution (I + G), and for ITS2 the model selected was Felsenstein 81 (F81; Felsenstein, 1981) with gamma distribution (G). The most appropriate substitution model for the combined data set was the same as for the COI gene alone, GTR + I + G.

Phylogenetic reconstructions based on the combined sequence data as well as on separate data from the two

mtDNA genes and the nuclear ITS2 region yielded trees with nearly identical topologies. However, because the resolution for trees based on the three regions separately (data not shown) was lower than for the trees from the combined data set, the latter were used to derive the final results. Phylogenetic trees obtained from MP and ML analyses for the combined data set are shown in Figs. 1 and 2, respectively. The MP consensus tree (Fig. 1) revealed two main clades with high bootstrap support values: clade A, comprising all the notional *C. acuminata* and *C. bignellii* haplotypes (82%); and clade B, including all the notional *C. melitaearum* haplotypes (100%). The ML analysis (Fig. 2) revealed the same two main clades, but with lower bootstrap support values.

In the MP tree, clade A was subdivided into four subclades, three of them well-supported (bootstrap values $\geqslant$80%). The four distinct subclades are: *C. acuminata* from host species *Euphydryas maturna* (Linnaeus) (A1); *C. acuminata* from *Melitaea phoebe* (Denis & Schiffermüller) and *Melitaea scotosia* Butler (A2); *C. bignellii* from *Euphydryas aurinia* (Rottemburg) (A3); and *C. acuminata* from host species *Melitaea didyma* (Esper) and *Melitaea latonigena* Eversmann (A4). The same clades were recovered in the ML tree (bootstrap support $\geqslant$90%), except that *C. acuminata* from *M. didyma* and *M. latonigena* did not group together. In the other major clade, clade B, *C. melitaearum* from several different host species and *C. cynthiae* group together comprising four subclades. The four distinct subclades are *Cotesia melitaearum* from *M. cinxia* (HT1 and HT2), *E. aurinia*, *E. aurinia davidi* (Oberthür) and *E. desfontainii* (Godart) (B1), *C. melitaearum* from *M. athalia* (Rottemburg), *M. deione* (Geyer) and *M. parthenoides* (Keferstein) (B2), *Cotesia cynthiae* from *Euphydryas cynthia* (Denis & Schiffermüller) (B3) and *C. melitaearum* from *M. didyma* and *M. trivia* (Denis & Schiffermüller) (B4). All these clades were revealed by both the MP tree (Fig. 1) and the ML tree (Fig. 2), but with

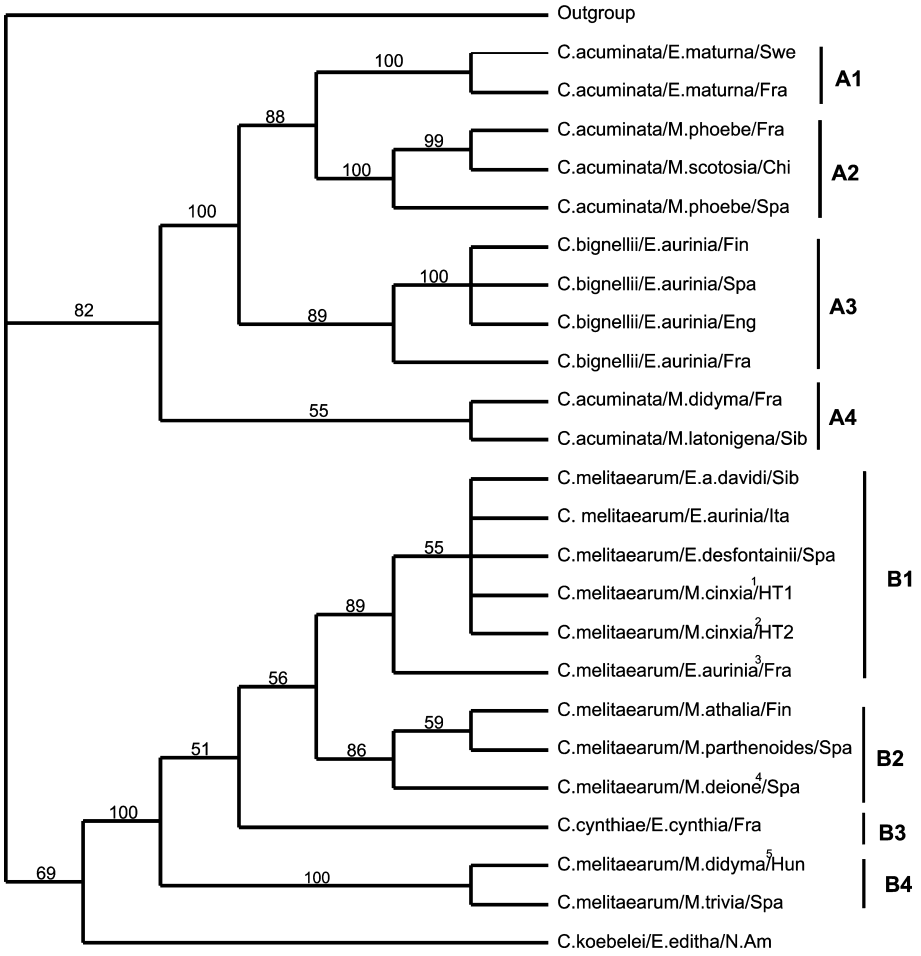

Fig. 1. Maximum parsimony tree of *Cotesia* species from different Melitaeini host species based on combined analysis of two mtDNA genes (COI and NDI) and nuclear region ITS2. Bootstrap support estimates (100 replicates) are indicated for statistically supported groupings ($\geqslant$50%). Vertical bars indicate the main clades (A,B) and the subclades (A1 etc., see text). The names of the *Cotesia* species, their host butterfly species and sampling localities are given in Appendix. (1) Haplotype "melitaearum/cinxia/HT1" occurs in Finland, Sweden, Estonia, England, France, Siberia, and China. (2) Haplotype "melitaearum/cinxia/HT2" occurs in France, Siberia and Spain. (3) Haplotype "melitaearum/aurinia/Fra" occurs also in Spain. (4) Haplotype "melitaearum/deione/Spa" occurs also in France. (5) Haplotype "melitaearum/didyma/Hun" occurs also in Spain.

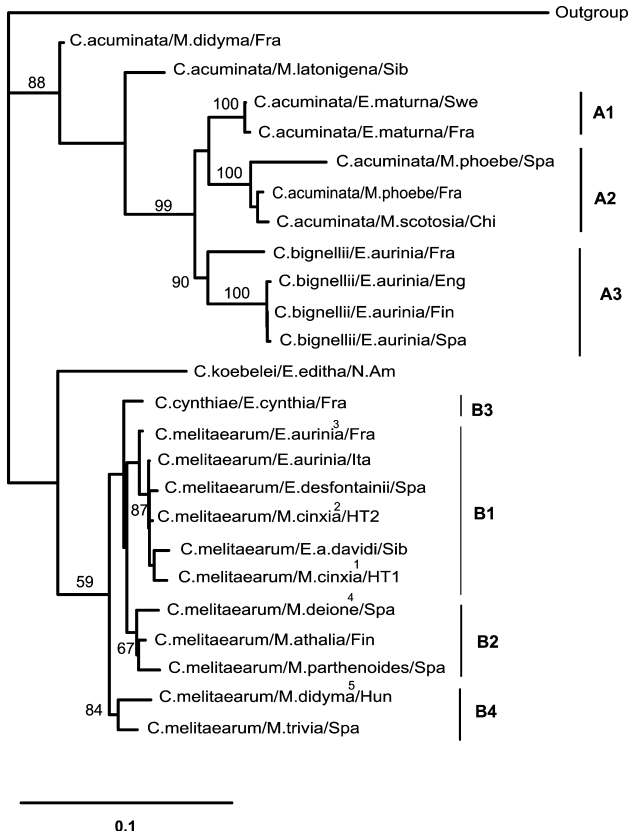

Fig. 2. Maximum likelihood distance tree of *Cotesia* species from different Melitaeini host species based on combined analysis of two mtDNA genes (COI and NDI) and nuclear region ITS2. Bootstrap support estimates (100 replicates) are indicated for statistically supported groupings (≥50%). Vertical bars indicate the main clades (A,B) and the subclades (A1 etc., see text). The names of the *Cotesia* species, their host butterfly species and sampling localities are given in Appendix. (1) Haplotype "melitaearum/cinxia/HT1" occurs in Finland, Sweden, Estonia, England, France, Siberia, and China. (2) Haplotype "melitaearum/cinxia/HT2" occurs in France, Siberia, and Spain. (3) Haplotype "melitaearum/aurinia/Fra" occurs also in Spain. (4) Haplotype "melitaearum/deione/Spa" occurs also in France. (5) Haplotype "melitaearum/didyma/Hun" occurs also in Spain.

higher and lower bootstrap support values, respectively. The North American species *Cotesia koebelei* from *Euphydryas editha* (Boisduval) grouped together with *C. melitaearum* and *C. cynthiae* in both MP and ML trees.

3.2. Microsatellites

The mean number of alleles among the microsatellite loci studied for *Cotesia* ranged from 1.0 to 4.3 (Appendix B). The number of alleles was highest, 15, at the locus *Cme15*. Average observed heterozygosity within populations varied from 0 to 0.45 and expected gene diversity (Nei, 1987) varied from 0 to 0.49 (Appendix B). Neighbour-joining trees calculated separately for the two main clades based on chord distance (D_{CE}) from the microsatellite data are shown in Figs. 3 and 4. *Cotesia congregata* was used as outgroup in both trees.

The phylogenetic tree constructed for clade A included one major clade including all *C. acuminata* and *C. bignellii* haplotypes except *C. bignellii* from *E. aurinia* from France, which remained unresolved at the base of the tree. The North American species *Cotesia euphydryidis* from *Euphydryas phaeton* (Drury) formed a distinct clade inside the major clade but outside the other *C. acuminata* and *C. bignellii* clades. The main clade was further divided into four subclades: *C. acuminata* from *E. maturna*, *M. scotosia* and from *M. phoebe* from France (A1 + A2 in the MP and ML trees); *C. bignellii* from *E. aurinia* (A3 in the MP and ML trees); *Cotesia acuminata* from *M. didyma*, *M. latonigena* and *M. athalia* (A4 in the MP and ML trees); and *Cotesia acuminata* from *M. phoebe* from Spain and the North American *C. koebelei* from *E. editha*, but this clade does not correspond with any of the subclades in the MP and ML trees. The phylogenetic tree constructed for clade B was subdivided into three subclades: *Cotesia melitaearum* from *M. cinxia*, *E. aurinia*, and *E. desfontainii* (B1 in the MP and ML trees); *Cotesia melitaearum* from *M. athalia*, *M. deione* and *M. parthenoides* (B2 in the MP and ML trees); and *Cotesia melitaearum* from *M. didyma* and *M. trivia*, and *C. cynthiae* (B3 + B4 in the MP and ML trees).

4. Discussion

4.1. Phylogenetic relationships in clades A and B

Phylogenetic analyses based on combined sequence data under MP and ML criteria (Figs. 1 and 2) produced trees with two main clades A and B. These clades were further separated into several subclades.

In clade A, *C. acuminata* from *E. maturna* from several locations represents a single haplotype in subclade A1, while in subclade A2 there are substantial pairwise nucleotide differences (up to 4%) between *C. acuminata* individuals collected from *M. phoebe* and *M. scotosia* from different geographical locations. This might be explained by the fact that our samples of *C. acuminata* from *E. maturna* represent only the European populations, while samples of *C. acuminata* from *M. phoebe* and *M. scotosia* represent also those from Asia. *Cotesia bignellii* from *E. aurinia* from Finland, Spain, and England have almost identical sequence haplotypes in subclade A3, while *C. bignellii* from *E. aurinia* from France shows a substantial divergence of 5% relative to the others, though it still groups with them. It appears that these *C. bignellii* from France have differentiated from the other sampled *C. bignellii* populations in other locations in Europe. The resolution of the status of *C. bignellii* from France requires further study with additional samples from several different locations. *Cotesia acuminata* from *M. didyma* and *M. latonigena* group

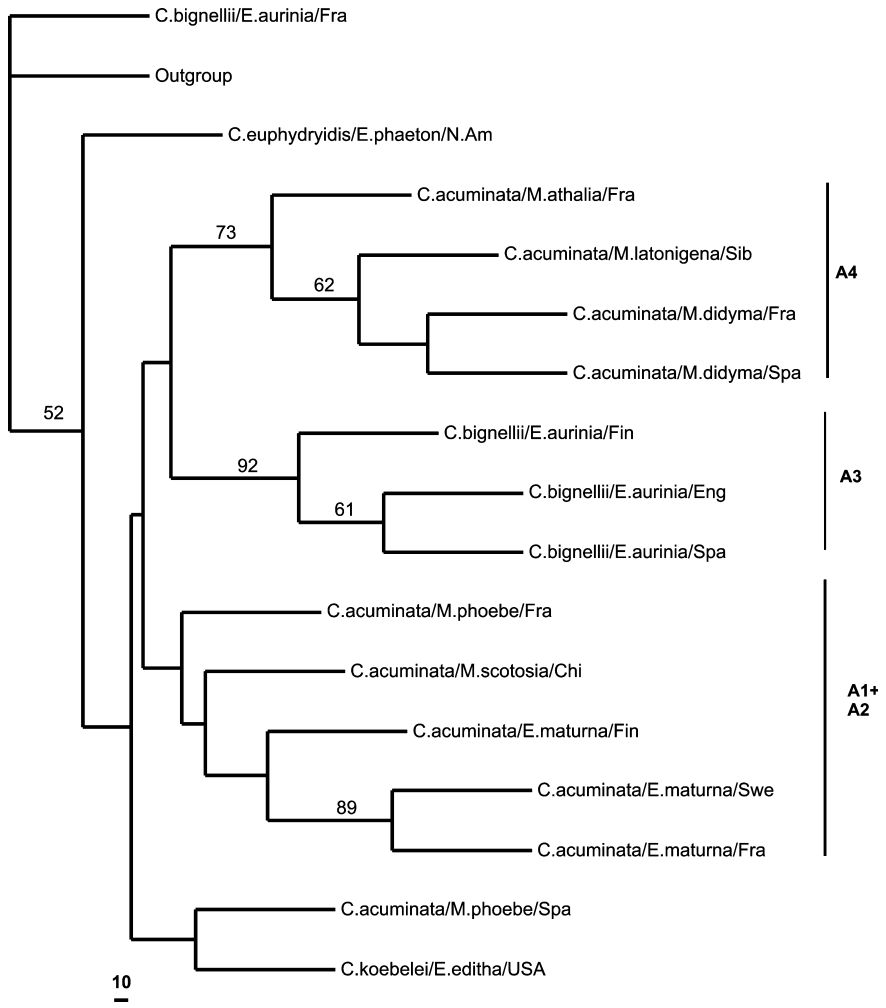

Fig. 3. Neighbour-joining tree calculated for the main clade A (="*Cotesia acuminata*" clade) from the microsatellite data. Distances are calculated based on the chord distance (D_{CE}) of Cavalli-Sforza and Edwards (1967) from 7 microsatellite loci. Bootstrap support estimates (100 replicates) are indicated for statistically supported groupings (⩾50%). Vertical bars indicate the subclades (see text). The names of the *Cotesia* species, their host butterfly species and sampling localities are given in Appendix A. Branch lengths are proportional to 10% as indicated by the scale.

together both in the MP tree and in the microsatellite tree (subclade A4), but they do differ substantially (up to 10%) from all the other *C. acuminata* and *C. bignellii* haplotypes in clade A, being actually more divergent from the remaining *C. acuminata* haplotypes than they are from *C. bignellii* haplotypes. As also all the three *C. acuminata* (A1, A2, A4) and one *C. bignellii* (A3) subclades are greatly divergent (4–10%) from each other, these results strongly suggest that *Cotesia acuminata* as currently recognised is not a single species but a group of probably two or three species, all showing a very high degree of host specificity. The morphological data amply support this in the case of the distinctive A4 (from *M. didyma* and *M. latonigena*), and much smaller but apparently consistent differences are also seen between A1 and A2 (Shaw, in prep.).

In the other major clade (B), *Cotesia melitaearum* haplotypes from *M. cinxia*, *E. aurinia*, *E. aurinia davidi*, and *E. desfontainii* group together in subclade B1 and *C. melitaearum* haplotypes from *M. athalia*, *M. deione* and *M. parthenoides* form subclade B2. *Cotesia cynthiae* is located inside the *C. melitaearum* clade and it comprises the subclade B3. *Cotesia melitaearum* haplotypes from *M. didyma* and *M. trivia* form the subclade B4. It seems probable that the types of *C. lycophron*, an enigmatic taxon known unequivocally only from the type series comprising a single brood reared from *M. didyma* in France, are aberrant specimens conspecific with the specimens of notional *C. melitaearum* subclade B4 from *M. didyma* (Shaw, in prep). All these results suggest that notional *C. melitaearum* is not a single species but a group of two or three species with very little consistent morphological differentiation.

4.2. Host specificity

Phylogenetic trees based on sequence data analysed with maximum parsimony and maximum likelihood, as

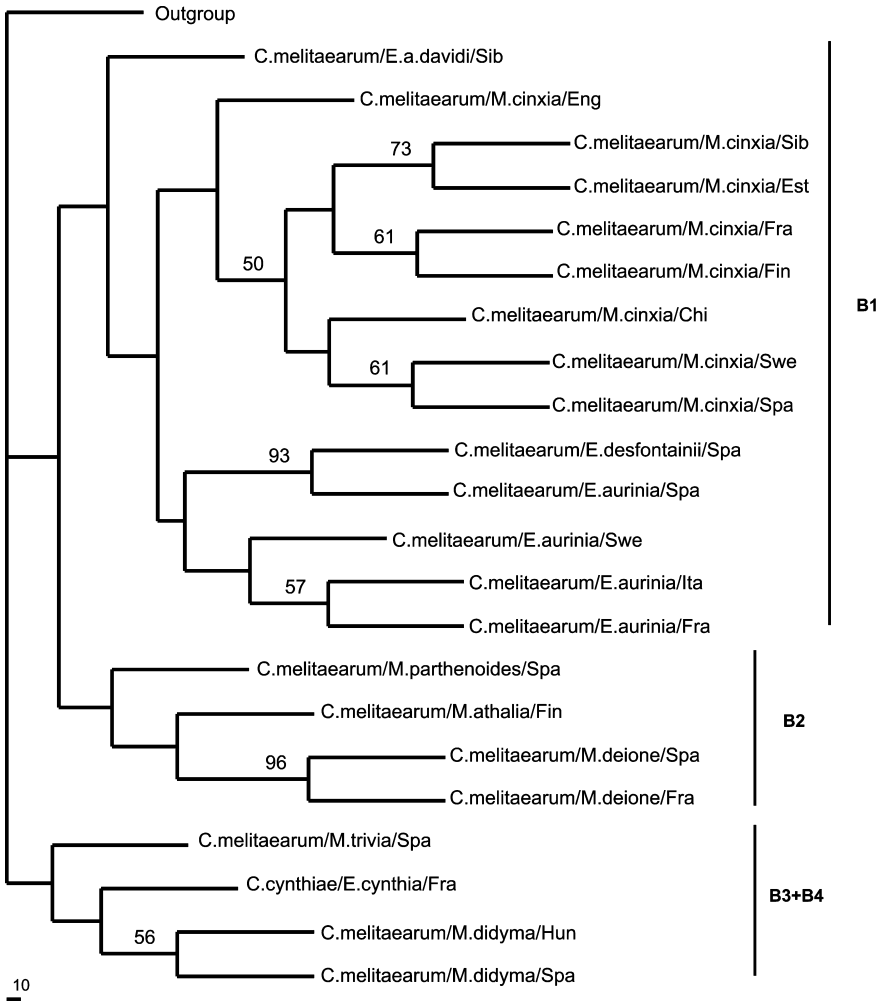

Fig. 4. Neighbour-joining tree calculated for the main clade B (= "*Cotesia melitaearum*" clade) from the microsatellite data. Distances are calculated based on the chord distance (D_{CE}) of Cavalli-Sforza and Edwards (1967) from 12 microsatellite loci. Bootstrap support estimates (100 replicates) are indicated for statistically supported groupings ($\geqslant 50\%$). Vertical bars indicate the subclades (see text). The names of the *Cotesia* species, their host butterfly species and sampling localities are given in Appendix A. Branch lengths are proportional to 10% as indicated by the scale.

well as NJ-trees based on microsatellite data, yielded a largely consistent picture of the evolutionary relationships among *Cotesia* parasitoids of Melitaeini. The most striking difference between the two major clades A and B relates to the host specificity of the parasitoids. The essential feature of clade A is the narrow host specialisation seen in the subclades in the sequence trees. The parasitoid segregates are either strictly monophagous (*C. bignellii* from *E. aurinia*, subclade A3) or involve allopatric sister-species pairs of hosts, as in subclade A4 in the case of *M. didyma* and *M. latonigena* (Wahlberg and Zimmermann, 2000) and subclade A2 in the case of *M. phoebe* and *M. scotosia* (Wahlberg and Zimmermann, 2000). To an extent host ranges may be found to be wider with more extensive sampling, but nevertheless it is clear that the taxa in clade A, which are generally common where they occur, do have genuinely narrow and non-overlapping host ranges.

In clade B, on the other hand, three of the four subclades parasitize several broadly sympatric host species. The exception is B4 consisting of *C. cynthiae* and parasitising *E. cynthiae* only; but this morphologically distinctive parasitoid is a specialised high-altitude species. The other difference between the two major clades is that in clade A some of the host species tend to live in different habitats (e.g., *M. phoebe* in open grasslands vs. *E. maturna* in woodland clearings and edges), which might have helped to maintain genetic isolation between diverging parasitoid populations, while in clade B they mostly live in flowery meadows. There is no simple pattern of host plant use in the different subclades. In our samples, four subclades (A1, A2, A4, and B1) occur both in Europe and Asia, while the rest are restricted to Europe only. However, our Asian samples are much more limited than samples from Europe (see Appendix A).

As indicated in the introduction, there is evidence that clade A and clade B do not constitute a monophyletic group, with the implication that the host group Melitaeini has probably been colonised twice by *Cotesia*. One might then ask which clade colonised Melitaeini

first. From a wide range of studies (e.g. Janz et al., 2001; and references therein; Liebherr and Hajek, 1990) it seems clear that there is no simple or generally applicable directionality in the evolution of the breadth of resource utilization by organisms, despite several potential mechanisms tending towards specialization as a derived trait (Futuyma and Moreno, 1988). However, extremely rapid divergence through shifts in ecology or invasion of new habitats have been demonstrated, and this might play a vital role in speciation through natural selection (Orr and Smith, 1998), presumably most often giving rise to nascent specialists. In some well-investigated groups of koinobiont parasitic Hymenoptera (Shaw, 1994, 2002; Shaw and Horstmann, 1997), a pattern of apparently closely related taxa having radically different breadths of host range has been seen. Those with the broadest host ranges often parasitize an array of phylogenetically unrelated but morphologically or behaviourally similar hosts occurring in the same microhabitat, and from these observations a process of niche-based host range expansion has been hypothesised as a prelude to speciation by specialization. In one group (the braconid genus *Aleiodes* Wesmael), in which host ranges have been best explored (Shaw, 1994, 2002 and unpublished), the capacity of some (presumably at one time nascent) specialists to remain specialised, rather than to engage in renewed host range expansion, can be deduced from the fact that the most morphologically isolated species (i.e., those apparently furthest from speciation events and thus lacking close relatives) are normally highly specialised to taxonomically narrow host ranges (Shaw, 2002). Although these ideas have not yet been rigorously tested, the alternative view that nascent species of koinobiont parasitic Hymenoptera first arise as generalists is untenable for other reasons (cf. Askew and Shaw, 1986). On this basis, from the narrowness of, and lack of overlap in, host ranges, and the greater degree of morphological differentiation between subclades, it seems that clade A has progressed further than clade B in the postulated process of colonisation, host range expansion by recruitment, and eventual fragmentation with competitive exclusions (and perhaps extinctions) then resulting in relatively isolated specialists. While this might imply that clade A (the "*acuminata*-group") was the first to colonise Melitaeini, it may merely reflect a higher evolutionary rate in clade A than in clade B (the "*melitaearum*-group"), in which the species are currently much less specialised, and may be evolving more actively. Interestingly, however, when taxa from these two clades use the same host species (e.g., *M. didyma*, *E. maturna*, and *E. aurinia*), the clade A species seem to be more successful. Clade B appears not to have been able to colonize *M. phoebe* at all, possibly because of the presence of a clade A specialist. If this suggests that the local survival of clade B species in the presence of clade A competitors will generally depend on the presence of additional "refuge" host species that are not used by the co-occurring clade A competitor, it may tend to inhibit processes of speciation through specialization in the clade B taxa.

4.3. Adaptations to Melitaeini as hosts

Generally, but to a variable extent (Kuussaari et al., 2004), Melitaeini as young larvae construct webs for the earliest part of their existence, especially as they feed towards and reach their winter diapause. Following diapause, and also in the case of plurivoltine species in non-diapausing generations, the tendency to live gregariously or to produce webbing is much diminished or absent. The larvae of *Euphydryas* species tend to have longer protective spines than larvae of *Melitaea* species, and also to use webbing more extensively. *Euphydryas* species are essentially univoltine (or have extended life cycles; Eliasson and Shaw, 2003) but in *Melitaea*, at least in warm temperate areas, there is a marked tendency to plurivoltism. Gregariousness and web building are thought to have evolved at least in part as a defence against natural enemies (Kuussaari et al., 2004; van Nouhuys and Hanski, 2004). *Cotesia* parasitoids of Melitaeini appear to be specialised to using gregarious hosts, and at least those that have been observed are not hindered from oviposition by the web (Lei and Camara, 1999; Stamp, 1981) and appear to benefit from large host group size (Lei and Camara, 1999; Porter, 1983; Stamp, 1981). These *Cotesia* species generally have two (or even three) generations per host generation, thus successively attacking host larvae of very different sizes, but always using hibernating host larvae in which to pass the winter themselves. The *Cotesia* adults do not, therefore, attack hosts only in webs, though in general they may depend on being able to do so at least for their own overwintering generation (but see Eliasson and Shaw, 2003).

The *Cotesia* species in clade A are characterised by having a longer and more pointed hypopygium and a somewhat projecting ovipositor, which presumably enables them to cope especially well with the most spinose hosts and with hosts living in webs and, indeed, this clade appears to be most strongly associated with *Euphydryas* species. The taxa of clade B, on the other hand, have a more truncate hypopygium and a less projecting ovipositor, suggesting an origin less adapted to the most spinose hosts and those making the most extensive use of webs, and many of the hosts used are *Melitaea* species. Further analysis is severely hampered by the lack of reliable and accurately comparative data on the precise habits and defensive behaviours of the early stages of all species of Melitaeini, by regional differences between populations of the same Melitaeini taxa, and by differences in behaviour between overwintering and non-overwintering host generations. Nevertheless there are good grounds for suspecting that different attributes of

the two *Cotesia* clades have resulted in different opportunities to permeate the host group overall, probably reflecting a difference in ancestral hosts.

In summary, our results show that phylogenetic trees based on sequence data analysed with maximum parsimony and maximum likelihood as well as NJ-trees contructed for microsatellite data lead to similar interpretations of the intraspecific and interspecific relationships of *Cotesia* parasitising Melitaeini. The Eurasian species comprise two distinct clades with a striking difference in the degree of host specificity and, also taking into account morphological and natural history data, some evolutionary scenarios can be suggested. It is clear that the current taxonomy with four species (or five including *C. lycophron*) does not agree with the phylogeny based on molecular data, which suggests the presence of several additional, previously unrecognised, *Cotesia* species.

Acknowledgments

We sincerely thank Ilkka Hanski for providing the initial idea for this work. The *Cotesia* samples used in the study were kindly provided by numerous collectors (in alphabetical order): Richard R. Askew (UK), Geoff N. Burton (UK), Peter W. Cribb (UK), Henry Decimon (France), Claes U. Eliasson (Sweden), Lei Guanchung (China), Ilkka Hanski (Finland), Juha Jantunen (Finland), Karen Kester (USA), Atte Komonen (Finland), Kathleene Jensen (USA), Gabriel Néve (France), Ulf Norberg (Sweden), Jim Pateman (UK), Joseph Planas (Spain), Donald L.J. Quicke (UK), Peter J.C. Russell (UK), Michael Singer (USA), Lee Slaughter (UK), Nancy Stamp (USA), Constantí Stefanescu (Spain), Peter Summers (UK), Roger Sutton (UK), Saskya van Nouhuys (USA), Veli Vikberg (Finland), Niklas Wahlberg (Sweden), Brian Wee (USA), and Jim B. Whitfield (USA). We warmly thank Toshka Nyman for help in the laboratory. Comments from Oscar Gaggiotti, Ilkka Hanski, Gavin Hinten, Tytti Kontula, Pierre-Alexandre Landry, Saskya van Nouhuys, Niklas Wahlberg, and two anonymous referees helped to improve earlier versions of the manuscript. The study has been financially supported by Academy of Finland (Grant Nos. 38604 and 44887 to Ilkka Hanski; Finnish Centre of Excellence Programme (2000–2005)).

Appendix A

Cotesia samples, host butterfly species and localities

Notional species	Host species*	Country	Locality	# Ind.	Collectors
C. acuminata	*Melitaea scotosia*	China	Peking	10	I.H., L.G.
C. acuminata	*Euphydryas maturna*	Finland	Joutseno, Tiuruniemi	3	A.K.
C. acuminata	*Euphydryas maturna*	France	Côte d'Or, Moloy	3	P.J.C.R.
C. acuminata	*Euphydryas maturna*	Sweden	Västmanland, Lindesberg	5	C.U.E.
C. acuminata	*Melitaea athalia*	France	Var, St. Paul-en-Fôret	2	P.W.C.
C. acuminata	*Melitaea didyma*	France	Vaucluse, La Roque sur Pernes	1	M.R.S.
			Bouches du Rhone, Aubagne	5	G.N.
			Pyrènèes Orien., Prats de Mollo	1	C.S.
C. acuminata	*Melitaea didyma*	Spain	Girona, Cantallops	12	C.S.
			Barcelona, El Cortès	3	C.S.
C. acuminata	*Melitaea latonigena*	Russia	Siberia, Buryatia	5	N.W., I.H.
C. acuminata	*Melitaea phoebe*	France	Dordogne, Ste Foy	2	R.R.A.
			Côte d'Or, Lusigny-sur-Ouche	2	P.J.C.R.
			Var, Draguignan	5	M.R.S.
C. acuminata	*Melitaea phoebe*	Spain	Barcelona, El Puig	15	C.S., J.P*
			Barcelona, Font Borrell	3	C.S.
			Barcelona, La Nou de Bergueda	1	C.S.
C. bignellii	*Euphydryas aurinia*	England	Dorset, Verwood	5	P.S.
C. bignellii	*Euphydryas aurinia*	Finland	Joutseno, Tiuriniemi	6	A.K.
			Joutseno, Tiuriniemi	3	J.J.
C. bignellii	*Euphydryas aurinia*	France	Laus de Cervieres	5	M.S.
			Dordogne, Mezieres	4	R.R.A.
C. bignellii	*Euphydryas aurinia*	Spain	Catalonia, La Baraca	6	M.S.
C. cynthiae	*Euphydryas cynthia*	France	Laus de Cervieres	6	M.S.
C. euphydryidis	*Euphydryas phaeton*	USA	Warren, 1987, Front Royal	5	N.S.
C. koebelei	*Euphydryas editha*	USA	YNP, Dana Meadows	2	M.S., B.W.
			YNP, Mount Dana	1	M.S., B.W.
			YNP, Parker pass	2	M.S., B.W.
C. melitaearum	*Euphydryas aurinia*	France	Marseilles, Bouches du Rhone	7	A.K.
C. melitaearum	*Euphydryas aurinia*	Italy	San Remo	6	H.D.

Appendix A (*continued*)

Notional species	Host species*	Country	Locality	# Ind.	Collectors
C. melitaearum	*Euphydryas aurinia*	Spain	Barcelona, Coll d' Estenalles	1	M.S.
			Catalonia, La Barroca	4	M.S.
			Barcelona, Bosc de Valldemaria	4	M.S.
			Barcelona, La Malesa	1	M.S.
			Barcelona, El Guix	12	C.S.
			Barcelona, Alzinar de Sant Marti	4	C.S.
C. melitaearum	*Euphydryas aurinia*	Sweden	Västmanland, Nora	2	M.R.S.
C. melitaearum	*Euphydryas a. davidi*	Russia	Siberia, Ivolginsk	6	N.W., I.H.
C. melitaearum	*Euphydryas desfontainii*	Spain	Barcelona, La Malesa	2	C.S., J.P.
			Barcelona, Boixadors	3	C.S., J.P.
			Barcelona, El Guix	9	C.S., J.P.
			Bergueda, Font Negra	2	C.S., J.P.
C. melitaearum	*Melitaea athalia*	Finland	Åland, patch 584	3	G.L., S.vN.
			Åland, patch 860	2	G.L., S.vN.
C. melitaearum	*Melitaea cinxia*	Estonia	Kaugatuma	23	S. vN.
			Karala	1	S. vN.
			Kogula	2	S. vN.
C. melitaearum	*Melitaea cinxia*	Finland	Åland, 32 patches	151	G.L., S.vN.
C. melitaearum	*Melitaea cinxia*	France	Pyrenees	5	A.K.
			Massigneu, Alps	7	A.K.
			Montpellier, Mortes	5	A.K.
			Marseilles, Bouches du Rhone	6	A.K.
			Alps, Billieme	1	A.K.
C. melitaearum	*Melitaea cinxia*	Russia	Siberia, Step	2	I.H., N.W.
			Siberia, Djirga	2	I.H., N.W.
			Siberia, Ubukun	13	I.H., N.W.
			Siberia, Utalnskuga	2	I.H., N.W.
			Siberia, Utitzicina	5	I.H., N.W.
			Siberia, place 2	4	I.H., N.W.
C. melitaearum	*Melitaea cinxia*	Sweden	Öland	5	U.N.
C. melitaearum	*Melitaea cinxia*	England	Isle of Wight	2	M.R.S.
C. melitaearum	*Melitaea cinxia*	China	Peking	2	I.H.
C. melitaearum	*Melitaea cinxia*	Spain	Barcelona, El Puig	2	C.S.
C. melitaearum	*Melitaea didyma*	Hungary	Vas, Örség	4	M.R.S.
C. melitaearum	*Melitaea didyma*	Spain	Girona, Cantallops	3	C.S.
			Barcelona, El Cortés	2	C.S.
C. melitaearum	*Melitaea parthenoides*	Spain	Girona, Vall Ter	2	C.S.
C. melitaearum	*Melitaea trivia*	Spain	Leon, Cremenes	3	J.P.
			Barcelona, El Puig	10	C.S.
C. melitaearum	*Melitaea deione*	France	Gard, St Martial	5	G.N.B.
C. melitaearum	*Melitaea deione*	Spain	Barcelona, Vidra/Vallforners	13	C.S., J.P*
			Barcelona, Sant Bernat	8	C.S.
			Barcelona, La Pedrera	1	C.S.
			Barcelona, Corrals d'en Perera	1	C.S.
			Barcelona, Sant Maral	3	C.S.
Outgroups:					
Microplitis xanthopus	?	England	?	1	D.L.J.Q.
C. congregata	*Manduca q.*[a]	USA	Richmond area/Tobacco	1	K.J., K.K.
	Manduca q.[a]	USA	Blackstone/Tobacco	1	K.J., K.K.
	Ceratomia catalpae (Boisduval)	USA	Charlottesmille/Catalpa	1	K.J., K.K.
	Ceratomia catalpae (Boisduval)	USA	Richmond area/Catalpa	1	K.J., K.K.
Tot.				491	

Collectors: R.R.A., Richard R. Askew; G.N.B., Geoff N. Burton; P.W.C., Peter W. Cribb; H.D., Henry Decimon; C.U.E., Claes U. Eliasson (1991); L.G., Lei Guanchung; I.H., Ilkka Hanski; J.J., Juha Jantunen; K.K., Karen Kester; A.K., Atte Komonen; K.J., Kathleene Jensen; G.N., Gabriel Néve; U.N., Ulf Norberg; J.P., Jim Pateman; J.P.*, Joseph Planas; D.L.J.Q., Donald L.J. Quicke; P.J. C.R., Peter J.C. Russell; M.R.S., Mark R. Shaw; M.S., Michael Singer; L.S., Lee Slaughter; N.S., Nancy Stamp; C.S., Constantí Stefanescu; P.S., Peter Summers; R.S., Roger Sutton; S.vN., Saskya van Nouhuys; N.W., Niklas Wahlberg; B.W., Brian Wee.

[a] quinquemaculata.

* Names of the Melitaeine host species according to Wahlberg (2000).

Appendix B

Microsatellite diversity estimates of different *Cotesia* haplotypes

Haplotype	Sample size[a]	Loci typed[b]	He	SD	Ho	SD	Alleles*	Allele range[c]
acuminata/maturna/Fin	3	6	0.250	0.250	0.250	0.217	1.2	1–2
acuminata/maturna/Fra	3	7	0.195	0.130	0.238	0.100	1.4	1–3
acuminata/maturna/Swe	5	6	0.093	0.093	0.100	0.055	1.2	1–2
acuminata/phoebe/Fra	9	7	0.200	0.200	0.200	0.179	1.6	1–3
acuminata/phoebe/Spa	19	8	0.267	0.117	0.275	0.045	2.6	1–8
acuminata/scotosia/Chi	10	9	0.000	0.000	0.000	0.000	1.1	1–2
bignellii/aurinia/Fin	9	11	0.336	0.107	0.137	0.051	2.2	1–4
bignellii/aurinia/Fra	9	10	0.278	0.081	0.171	0.051	1.9	1–3
bignellii/aurinia/Spa	6	11	0.179	0.101	0.159	0.063	1.3	1–2
bignellii/aurinia/Eng	5	9	0.111	0.111	0.111	0.105	1.1	1–2
acuminata/athalia/Fra	2	9	0.333	0.167	0.333	0.157	1.3	1–2
acuminata/didyma/Fra	6	6	0.127	0.078	0.067	0.048	1.5	1–2
acuminata/didyma/Spa	16	6	0.087	0.087	0.167	0.040	1.2	1–2
acuminata/latonigena/Sib	5	7	0.219	0.150	0.238	0.114	1.3	1–2
melitaearum/a.davidi/Sib	6	12	0.323	0.082	0.408	0.067	1.9	1–3
melitaearum/aurinia/Fra	7	12	0.367	0.097	0.200	0.052	2.3	1–4
melitaearum/aurinia/Ita	6	12	0.402	0.088	0.254	0.058	2.3	1–4
melitaearum/aurinia/Spa	24	11	0.403	0.106	0.280	0.035	4.0	1–13
melitaearum/aurinia/Swe	2	11	0.348	0.106	0.318	0.099	1.7	1–3
melitaearum/desfontainii/Spa	16	11	0.443	0.114	0.288	0.051	3.5	1–10
melitaearum/cinxia/Fin	151	12	0.307	0.097	0.148	0.013	4.0	1–15
melitaearum/cinxia/Chi	2	12	0.042	0.042	0.042	0.042	1.1	1–2
melitaearum/cinxia/Est	26	12	0.325	0.062	0.173	0.040	2.6	1–6
melitaearum/cinxia/Fra	30	12	0.394	0.107	0.252	0.029	4.0	1–13
melitaearum/cinxia/Sib	29	12	0.485	0.088	0.390	0.037	4.3	1–11
melitaearum/cinxia/Spa**	2	11	—	—	—	—	1.0	1–1
melitaearum/cinxia/Swe**	5	12	—	—	—	—	1.3	1–2
melitaearum/cinxia/Eng	2	10	0.233	0.100	0.200	0.089	1.5	1–3
melitaearum/athalia/Fin	5	11	0.375	0.095	0.305	0.064	2.0	1–3
melitaearum/parthenoides/Spa	2	9	0.296	0.117	0.444	0.117	1.4	1–2
melitaearum/deione/Fra	5	10	0.278	0.096	0.240	0.060	1.8	1–3
melitaearum/deione/Spa	26	11	0.260	0.066	0.242	0.028	2.0	1–3
cynthiae/cynthia/Fra	6	10	0.000	0.000	0.000	0.000	1.1	1–2
melitaearum/didyma/Hun	4	10	0.327	0.090	0.450	0.082	1.9	1–4
melitaearum/didyma/Spa	5	10	0.464	0.113	0.391	0.067	2.7	1–4
melitaearum/trivia/Spa	13	10	0.250	0.093	0.256	0.046	1.9	1–3
euphydryidis/phaeton/N.Am	5	6	0.219	0.108	0.275	0.083	1.7	1–3
koebelei/editha/N.Am	5	8	0.388	0.103	0.250	0.082	2.3	1–3

[a] Number of individuals.

[b] Loci analysed.

[c] Allele range over all loci are calculated from all the individuals. Observed heterozygosity (H_0) and expected gene diversity (H_E) and standard deviations for HE, and H0 are calculated from females (see text).

* Mean number of alleles over all loci.

** No females available.

References

Askew, R.R., Shaw, M.R., 1986. Parasitoid communities: their size, structure and development. In: Wagge, J., Greathead, D. (Eds.), Insect Parasitoids. Academic Press, London, pp. 225–264.

Cavalli-Sforza, L.L., Edwards, A.W.F., 1967. Phylogenetic analysis: Models and estimation procedures. Am. J. Hum. Genet. 19, 233–257.

Eliasson, C.U., 1991. Studier av boknäftjärelens *Euphydryas maturna* (Lepidoptera: Nymphalidae) förekomst och biologi i Västmanland. Ent. Tidskr. 112, 113–124.

Eliasson, C.U., Shaw, M.R., 2003. Prolonged life cycles, oviposition sites, foodplants and *Cotesia* parasitoids of Melitaeini butterflies in Sweden. Oedippus 21, 1–52.

Erlich, P.R. & Hanski, I. (Eds.), 2004. On the wings of checkerspots: A model system in population biology, Oxford University Press, Oxford (in press).

Erlich, P.R., White, R.R., Singer, M.C., McKechnie, S.W., Gilbert, L.E., 1975. Checkerspot butterflies: a historical perspective. Science 188, 221–228.

Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. 17, 368–376.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using bootstrap. Evolution 339, 783–791.

Felsenstein, J., 1995. PHYLIP (Phylogenetic Inference Package), Version 3.57c. Department of Genetics, University of Washington, Seattle, WA.

Folmer, O., Black, M.B., Hoch, W., Lutz, R.A., Vrijehock, R.C., 1994. DNA primers for amplification of mitochondrial cytochrome *c*

oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.
Futuyma, D.J., Moreno, G., 1988. The evolution of ecological specialization. Annu. Rev. Ecol. Syst. 19, 207–233.
Ford, H.D., Ford, E.B., 1930. Fluctuation in numbers, and its influence on variation, in *Melitaea aurinia*, Rott. (Lepidoptera). Trans. Entomol. Soc. Lond. 78, 345–351.
Goldstein, D.B., Ruiz-Linares, A., Cavalli-Sforza, L.L., Feldman, M.W., 1995. An evaluation of genetic distances for use with microsatellite loci. Cenetics 139, 463–471.
Haeselbarth, E., 1979. Zur Parasitierung der Puppen von Forleule (*Panolis flammea* (Schiff.)), Kiefernspanner (*Bupalus piniarius* (L.)) und Heidelbeerspanner (*Boarmia bistortata* (Goeze)) in bayerischen Kiefernwäldern. Z. Angew. Entomol. 87, 186–202, 311–322.
Hanski, I., 1999. Metapopulation Ecology. Oxford University Press, Oxford.
Higgins, L.G., 1981. A revision of *Phyciodes* Hübner and related genera, with review of the classification of the Melitaeina (Lepidoptera: Nymphalidae). Bull. Br. Mus. Nat. Hist. (Ent.) 43, 77–243.
Janz, N., Nyblom, K., Nylin, S., 2001. Evolutionary dynamics of host-plant specialization: a case study of the tribe Nymphalini. Evolution 55 (4), 783–796.
Jensen, M.K., Kester, K.M., Kankare, M., Brown, B.L., 2002. Characterization of microsatellite loci of parasitoid, *Cotesia congregata* (Say) (Hymenoptera). Mol. Ecol. Notes 2, 346–348.
Kambhampati, S., Smith, P.T., 1995. PCR primers for the amplification of four insect mitochondrial gene fragments. Insect Mol. Biol. 4, 233–236.
Komonen, A., 1997. The parasitoid complex attacking Finnish populations of two threatened butterflies, *Euphydryas maturna* and *E. aurinia*. Baptria 22, 105–109.
Komonen, A., 1998. Host species used by parasitoids of Melitaeini in southern France. Baptria 22, 87–93.
Kuussaari, M., van Nouhuys, S., Hellmann, J., Singer, M.C., 2004. Larval biology of checkerspot butterflies. In: Erlich, P.R., Hanski, I. (Eds.), On The Wings of Checkerspots: A Model System in Population Biology. Oxford University Press, Oxford (in press).
Landry, P.-A., Koskinen, M.T., Primmer, C.R., 2002. Deriving evolutionary relationships with microsatellites and $(\delta\mu)^2$: all loci are equal, but some are more equal than others.... Genetics 161, 1339–1347.
Lei, G.C., Vikberg, V., Nieminen, M., Kuussaari, M., 1997. The parasitoid complex attacking the Finnish populations of Glanville fritillary *Melitaea cinxia* (Lep: Nymphalidae), an endangered butterfly. J. Nat. Hist. 31, 635–648.
Lei, G.C., Hanski, I., 1997. Metapopulation structure of *Cotesia melitaearum*, a specialist parasitoid of the butterfly *Melitaea cinxia*. Oikos 78, 91–100.
Lei, G.C., Camara, M.D., 1999. Behaviour of a specialist parasitoid, *Cotesia melitaearum*: from individual behaviour to metapopulation processes. Ecol. Ent. 24, 59–72.
Liebherr, J.C., Hajek, A.E., 1990. A cladistic test of the taxon cycle and taxon pulse hypotheses. Cladistics 6, 39–59.
Marsh, P.M., 1979. Braconidae. In: Krombein, K.V., Hurd, P.D., Smith, D.R., Burk, B.D. (Eds.), Catalog of Hymenoptera in America north of Mexico. Symphyta and Apocrita (Parasitica), vol. 1. Smithsonian Institute, Washington, DC, pp. 144–295.
Mason, W.R.M., 1981. The polyphyletic nature of *Apanteles* Foerster (Hymenoptera: Braconidae): a phylogeny and reclassification of Microgastrinae. Mem. Entomol. Soc. Can. No. 115, pp. 147.
Moore, S.D., 1989. Patterns of juvenile mortality within an oligophagous insect population. Ecology 70, 1726–1737.
Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York, NY.
Nixon, G.E.J., 1974. A revision of the north-western European species of the *glomeratus*-group of *Apanteles* Förster (Hymenoptera, Braconidae). Bull. Entomol. Res. 64, 453–524.
Ohta, T., Kimura, K., 1973. The model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a genetic population. Genet. Res. 22, 201–204.
Orr, M.R., Smith, T.B., 1998. Ecology and speciation. Trends Ecol. Evol. 13, 502–506.
Papp, J., 1986. A survey of the European species of *Apanteles* Först. (Hymenoptera, Braconidae: Microgastrinae). IX. The *glomeratus*-group 1. Ann. Hist-Nat. Mus. Nat. Hung. 78, 225–247.
Papp, J., 1987. A survey of the European species of *Apanteles* Först. (Hymenoptera, Braconidae: Microgastrinae). X. The *glomeratus*-group, 2 and the *cultellatus*-group. Ann. Hist-Nat. Mus. Nat. Hung. 79, 207–258.
Porter, K., 1981. The population dynamics of small colonies of the butterfly *Euphydryas aurinia*. D. Phil thesis, University of Oxford.
Porter, K., 1983. Multivoltinism in *Apanteles bignellii* and the influence of weather on synchronisation with its host *Euphydryas aurinia*. Entomol. Exp. Appl. 34, 155–162.
Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Rodreguez, F., Oliver, J.F., Mar, A., Medina, J.R., 1990. The general stochastic model of nucleotide substitutions. J. Theor. Biol. 142, 485–501.
Shaw, M.R., Huddleston, T., 1991. Classification and biology of braconid wasps (Hymenoptera: Braconidae). Handbooks for the Identification of British Insects 7(11), Royal Entomological Society of London, pp. 126.
Shaw, M.R., 1994. Parasitoid host ranges. In: Hawkins, B.A., Sheehan, W. (Eds.), Parasitoid Community Ecology. Oxford University Press, Oxford, pp. 111–144.
Shaw, M.R., Horstmann, K., 1997. An analysis of host range in the *Diadegma nanus* group of parasitoids in Western Europe, with a key to species (Hymenoptera: Ichneumonidae: Campopleginae). J. Hym. Res. 6, 273–296.
Shaw, M.R., 2002. Host ranges of *Aleiodes* species (Hymenoptera: Braconidae), and an evolutionary hypothesis. In: Melika, G., Thuróczy, C. (Eds.), Parasitic Wasps: Evolution, Systematics, Biodiversity and Biological Control. Agroinform, Budapest, pp. 322–327.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., Flook, P., 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87, 651–701.
Slatkin, M., 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139, 457–462.
Stamp, N.E., 1981. Effect of group size on parasitism in a natural population of the Baltimore Checkerspot *Euphydryas phaeton*. Oecologia 49, 201–206.
Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). 4.0b10. Sinauer Associates, Sunderland, MA.
Takezaki, N., Nei, M., 1996. Genetic distances and recontruction of phylogenetic trees from microsatellite data. Genetics 144, 389–399.
Thomas, C.D., Singer, M.C., 1998. Scale-dependent evolution of specialization in a checkerspot butterfly: from individuals to metapopulations and ecotypes. In: Mopper, S., Strauss, S.Y. (Eds.), Genetic Structure and Local Adaptation in Natural Insect Populations. Chapman and Hall, New York, NY, pp. 343–374.
Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.
van Nouhuys, S., Hanski, I., 2004. Natural enemies of checkerspot butterflies. In: Erlich, P.R., Hanski, I. (Eds.), On the wings of checkerspots: a model system in population biology. Oxford University Press, Oxford (in press).
van Veen, F.J.F., Belshaw, R., Godfray, H.C.J., 2003. The value of the ITS2 region for the identification of species boundaries between

Alloxysta hyperparasitoids (Hymenoptera: Charipidae) of aphids. Eur. J. Entomol. 100, 449–453.

Wahlberg, N., 2000. The ecology and evolution of melitaeine butterflies. PhD thesis, University of Helsinki, Finland.

Wahlberg, N., Zimmermann, M., 2000. Pattern of phylogenetic relationships among members of the tribe Melitaeini (Lepidoptera: Nymphalidae) inferred from mtDNA sequences. Cladistics 16, 347–363.

Wahlberg, N., Kullberg, J., Hanski, I., 2001. Natural History of some Siberian melitaeine butterfly species (Melitaeini: Nymphalidae) and their parasitoids. Ent. Fenn. 12, 72–77.

Warren, M.S., 1987. The ecology and conservation of the heath fritillary butterfly *Mellicta athalia*. J. Appl. Ecol. 24, 499–513.

White, R.R., 1973. Community relationships of the butterfly *Euphydryas editha*. PhD thesis. Stanford University.

Williams, E.H., Holdren, C.E., Ehrlich, P.R., 1984. The life history and ecology of *Euphydryas gillettii* Barnes (Nymphalidae). J. Lepid. Soc. 38, 1–12.

Yang, Z., 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. J. Mol. Evol. 39, 306–314.