



Phylogeny of *Maculinea* blues (Lepidoptera: Lycaenidae) based on morphological and ecological characters: evolution of parasitic myrmecophily

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Abstract

A phylogeny of blue butterflies of the genus *Maculinea* and related genera (Lycaenidae) is proposed, based on 91 morphological and ecological characters. The resulting tree shows that: (1) *Phengaris* is a derived group nested within *Maculinea*; (2) the *Maculinea-Phengaris* clade is probably nested within *Glaucopsyche*; (3) there are three well supported groups within the *Maculinea-Phengaris* clade: (*alcon* group ((*teleius* group) (*arion-Phengaris* group))). Some species (*M. alcon*, *M. arionides*) appear to be non-monophyletic and require reclassification. The two alternative strategies of parasitic myrmecophily in the *Maculinea-Phengaris* clade, viz., “predatory” and “cuckoo”, seem to be derived characters of the *alcon* group, and of the *teleius* and *arion-Phengaris* groups, respectively. The common ancestor of *Maculinea* used dorsal nectary organ secretions for ant attraction, while this trait was reduced in the ancestor of the *alcon* group and in *M. nausithous* (of the *teleius* group). The three recent *Maculinea* lineages utilize taxonomically diverse host plants, the asterid families Gentianaceae (*alcon* and *arion-Phengaris* groups), Lamiaceae (*arion-Phengaris* group), Campanulaceae (*arion-Phengaris* group), and the rosid family Rosaceae (*teleius* group).

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The blues of the genus *Maculinea* Van Eecke, 1915 (Lepidoptera: Lycaenidae) belong to the most intensively studied butterflies in Eurasia. In contrast to the vast majority of myrmecophilous lycaenid butterflies who receive protection from ants, *Maculinea* species have evolved an intricate form of nest parasitism. Their early larval instars are mono- to oligophagous feeders on flowers and developing seeds of herbs, but later parasitize the nests of ant genera *Myrmica* Latreille, 1804, and *Aphaenogaster* Mayr, 1853 (see, e.g., Fiedler, 1991; Wardlaw et al., 2000). This ecological complex has attracted much research on butterfly myrmecophily (e.g., Hochberg et al., 1994; Fiedler, 1998; Akino et al., 1999; Pierce et al., 2002). *Maculinea* have also been intensively studied by conservation biologists, since their specialized habits render them vulnerable to habitat alteration; they have exhibited severe declines in Europe (Wynhoff, 1998a; Van Swaay and Warren, 1999). The

extinction of *Maculinea arion* (Linnaeus, 1758) in the UK in the 1970s (Thomas, 1980) has in fact sparked interest in the scientific conservation and habitat restoration of butterflies (e.g., Elmes and Thomas, 1992; Hochberg et al., 1994; Thomas, 1994; Wynhoff, 1998b). It is therefore rather odd that there is still a lack of knowledge about phylogenetic relationship within the genus. This complicates our understanding of the origin and evolution of *Maculinea* parasitic development (Elmes et al., 2001; Als et al., 2002). It also causes controversies in terms of the taxonomic status of individual taxa, including those with a high conservation priority. Specifically, there are controversies concerning the status of *M. alcon* (Denis & Schiffermüller, 1775) and *M. rebeli* (Hirschke, 1904), two threatened European taxa, which spend the early phases of their development on Gentianaceae plants (compare Sibatani et al., 1994; Kudrna, 2001).

Regarding parasitic myrmecophily, *Maculinea* species vary in two conspicuous traits: the adoption ritual, and

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the mode of larval feeding within the ant nests. Adoption may or may not include the use of secretions from the dorsal nectary organ (DNO) for enticing the attention of ants. While in ant nests, the larval feeding may either be: (1) “cuckoo” when the ants feed the caterpillars via regurgitation, or (2) “predatory” when the caterpillars prey on ant broods. Since the species of *Maculinea* that employ the DNO secretions during adoption tend to be predatory (Table 1), and DNO secretions play a major role in mediating mutualism with ants in the majority of related non-parasitic genera of lycaenid butterflies, the predatory habit was proposed as ancestral for the genus (Fiedler, 1998). Further arguments corroborating the claim that cuckoo caterpillars are derived, include a lower fitness cost imposed upon the ants by cuckoos, shorter adoption time, and a more elaborate communication between the cuckoo caterpillars and ants (Fiedler, 1990, 1998; Elmes et al.,

1991, 2002; Thomas and Elmes, 1998). In addition, several putative non-parasitic *Maculinea* outgroups feed on Lamiaceae, which are also utilized by the predatory species *M. arion* and *M. arionides*. Fiedler (1998) suggested that the ancestor of *Maculinea* evolved in high-altitude steppe-like habitats with a long unfavorable season, which forced it to spend part of its development within ant nests.

At this time, it is uncertain how closely individual species of *Maculinea* are associated with individual species of ants. The associations were considered highly specific by Thomas et al. (1989), but recent studies (e.g., Wardlaw et al., 1998; Steiner et al., 2003) have suggested that the situation is much more complex (Als et al., 2002). Therefore, we have refrained from including ant specificity characters in our phylogenetic analyses.

The purpose of this paper is to test the above hypotheses of the evolution of parasitic myrmecophily

Table 1

List of terminals used for the analysis, their host plant associations, geographic distribution, and associations with ants. Species investigated by present authors are presented in bold typeface

Taxon	Host plant	Geogr.	Ant assoc.	DNO secret.
<i>Lycaena dispar</i> (Haworth, 1803)	Polygonaceae	Pal	absent	no
<i>Polyommatus icarus</i> (Rottentburg, 1775)	Fabaceae	Pal	mutual	++
<i>Pseudophilotes vicrama</i> (Moore, 1865)	Lamiaceae	Pal	mutual	++
<i>Iolana iolas</i> (Ochsenheimer, 1816)	Fabaceae	WPal	mutual	++
<i>Shijimiaeoides divina</i> (Fixsen, 1887)	Fabaceae	EPal	?	?
<i>Sinia lanty</i> Oberthür, 1886	?	SEPal	?	?
<i>S. leechi</i> (Forster, 1940)	?	SEPal	?	?
<i>Caerulea coelestis</i> (Alphéraky, 1897)	?	SEPal	?	?
<i>Glaucopsyche piasus</i> (Boisduval, 1852)	Fabaceae	Nearct	mutual	?
<i>G. alexis</i> (Poda, 1761)	Fabaceae	WPal	mutual	++
<i>G. melanops</i> (Boisduval, 1828)	Fabaceae	WPal	mutual	++
<i>Phengaris atroguttata</i> (Oberthür, 1876)	Lamiaceae	SEPal	predat	?
<i>P. daitozana</i> Wileman, 1908	Gent.-Camp.	Taiwan	?	?
<i>P. albida</i> Leech, 1893	?	China	?	?
<i>Maculinea rebeli</i> (Hirschke, 1904)	Gentianaceae	Europe	cuckoo	+
<i>M. alcon alcon</i> (Dennis and Schiffermüller, 1775)	Gentianaceae	WPal	cuckoo	+
<i>M. alcon kondakovi</i> (Kurentsov, 1970)	Gentianaceae	EPal	?	?
<i>M. alcon arirang</i> Sibatani, Saigusa & Hirowatari, 1994	?	EPal	?	?
<i>M. arion arion</i> (Linnaeus, 1758)	Lamiaceae	WPal	predat	++
<i>M. arion xiaheana</i> (Murayama, 1919)	?	China	?	?
<i>M. arion ussuriensis</i> (Sheljuzhko, 1928)	?	EPal	?	?
<i>M. arion cyaneacula</i> (Eversmann, 1848)	?	CPal	?	?
<i>M. arion inferna</i> Sibatani, Saigusa & Hirowatari, 1994	?	Tibet	?	?
<i>M. arionides arionides</i> (Staudinger, 1887)	Lamiaceae	EPal	?	?
<i>M. arionides takamukui</i> (Matsumura, 1919)	Lamiaceae	Japan	predat	?
<i>M. nausithous</i> (Bergsträsser et al. [1779])	Rosaceae	WPal	ambig*	+
<i>M. kurentzovi</i> Sibatani, Saigusa & Hirowatari, 1994	Rosaceae	EPal	?	?
<i>M. teleius teleius</i> (Bergsträsser et al. [1779])	Rosaceae	WPal	predat	++
<i>M. teleius euphemia</i> (Staudinger, 1887)	Rosaceae	EPal	?	?
<i>M. teleius sinalcon</i> Murayama, 1992	?	China	?	?
<i>M. teleius daisensis</i> (Matsumura, 1926)	Rosaceae	Japan	?	?
<i>M. teleius hosonoi</i> Takahashi, 1973	Rosaceae	Japan	?	?
<i>M. teleius kazamoto</i> (Druce, 1875)	Rosaceae	Japan	?	?
<i>M. teleius ogumae</i> (Matsumura, 1910)	Rosaceae	Sakhalin	?	?

See Material and Methods; Host plant: Gent., Gentianaceae; Camp., Campanulaceae; Geogr., geographic distribution: Pal, Palearctics; Nearct, Nearctics; E, east; W, west; SE, south-east; C, central; Ant assoc., type of ant association: mutual, mutualistic; predat, predatory; DNO secret., dorsal nectary organ secretion: +, rare; ++, frequent.

in *Maculinea*, using a cladistic hypothesis based on morphological and ecological data, and to reconstruct the history of: (i) the use of DNO secretions during adoption, (ii) predatory versus cuckoo feeding, (iii) host plant use, and (iv) habitat preferences of individual species.

Materials and methods

Taxonomy

Maculinea is distributed across the Palaearctic region and consists of a minimum of seven species (Sibatani et al., 1994; Table 1). The most closely related genera include *Bajluana* Korshunov, 1990, *Caerulea* Forster, 1938, *Euphilotes* Mattoni, 1977; *Glaucopsyche* Scudder, 1872, *Iolana* Bethune-Baker, 1914, *Micropsyche* Mattoni [1981], *Otnjukovia* Zhdanko, 1997, *Paleophilotes* Forster, 1938, *Phengaris* Doherty, 1891, *Philotes* Scudder, 1876, *Philotiella* Mattoni, 1977; *Praepphilotes* Forster, 1938, *Pseudophilotes* Beuret, 1955, *Scolitantides* Hübner, 1819, *Shijimiaeoides* Beuret, 1955, *Sinia* Forster, 1940, *Subsulanooides* Koiwaya, 1989 (only provisionally placed here), and *Turanana* Bethune-Baker, 1916; all being classified as members of the “*Glaucopsyche*-section” of Lycaenidae: Polyommataini (Eliot, 1973; Mattoni, 1977, 1979[1981]). Hesselbarth et al. (1995) proposed the division of this section into two lineages: Scolitantiditi, including *Scolitantides* and *Pseudophilotes*; and Glaucopsychiti, including *Glaucopsyche* (*sensu lato*, i.e., including *Maculinea* and *Iolana*), *Turanana* and *Lachides*.

Given the confused state of *Maculinea* alpha-taxonomy, we refrained from resolving specific taxonomic controversies before selecting terminal taxa for the cladistic analysis and included the nominal taxa regardless their formal “species” or “subspecies” ranks. We focused on: (1) all the “established” European species, recognized in recent mainstream lepidopterist literature (e.g., Karsholt and Razowski, 1996; Wynhoff, 1998b) and (2) the Asian taxa revised by Sibatani et al. (1994) (Table 1).

In selecting outgroup taxa, we tried to represent the diversity of the *Glaucopsyche*-section by including: (i) some representatives of both of the lineages, Scolitantiditi and Glaucopsychiti, recognized by Hesselbarth et al. (1995); (ii) representatives of all genera that were considered congeneric with *Maculinea* by recent authors (e.g., Cano, 1981; Bálint, 1989); (iii) all three representatives of East-Asian *Phengaris*, the only other genus of the Glaucopsychiti exhibiting parasitic myrmecophily (Fiedler, 1998; Elmes et al., 2001; Pierce et al., 2002); (iv) *Polyommatus icarus* (Polyommataini) and *Lycaena dispar* (Lycaenini) as more distant lycaenid butterflies. The nomenclature and higher classification of

non-*Maculinea* taxa follow Eliot (1973) and Tolman and Lewington (1997).

Character sampling

The characters used in the phylogenetic analysis (for the list of characters see Appendix 1; for the data matrix Appendix 2) were extracted from literature, as well as from our study of the morphology of collection specimens (“orig.” hereinafter). The terminalia characters ($N = 37$; sources Hemming, 1931; Beuret, 1958; Shirozu, 1960; Agenjo, 1967; Bernardi, 1972; Eliot, 1973; Higgins, 1975; Kawazoe and Wakabayshi, 1976; Mattoni, 1977; Mattoni and Fiedler, 1991; Sibatani et al., 1994; Jaksic, 1998; orig.) were coded according to Sibatani et al. (1994). Wing color pattern traits ($N = 34$; from Shirozu, 1960; Kurentsov, 1970; Kawazoe and Wakabayshi, 1976; Inomata, 1990; D’Abrera, 1993; Sibatani et al., 1994; Korshunov and Gorbunov, 1995; Tuzov et al., 2000; Berlov, 2001; orig.) were coded using a combination of the “Nymphalid Ground Plan”, a diagrammatic system for identifying individual pattern elements across the Lepidoptera (Nijhout, 1991), and the codes for wing spots shown in Fig. 1. According to numerous morphological, developmental, and genetic studies (for recent reviews see Beldade et al., 2002;

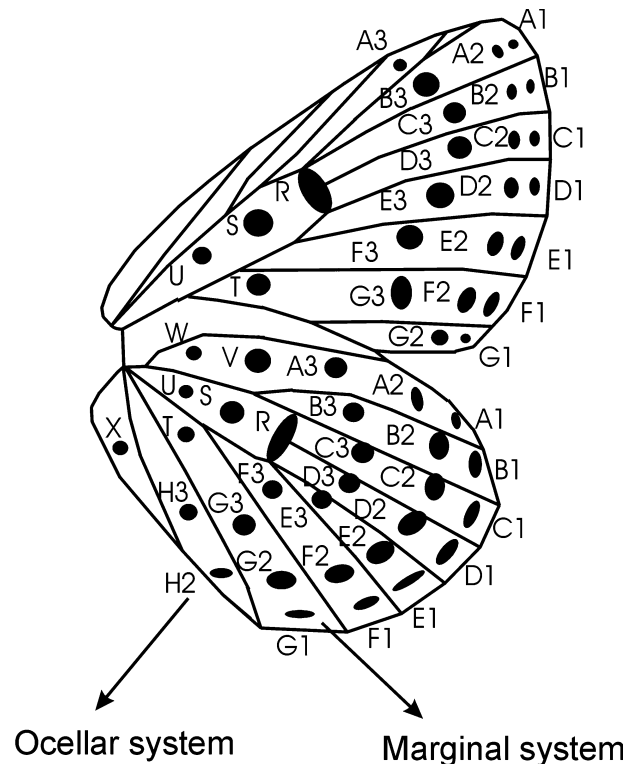


Fig. 1. System of coding of wing pattern traits used in the phylogenetic study of *Maculinea* and their relatives.

Brakefield, 2003) the individual wing pattern elements evolve in a more or less independent manner, which justifies treating them as separate characters in the cladistic analyses. Additional characters concerned the eyes ($N = 1$; Higgins, 1975; orig.), labial palps ($N = 2$; Mattoni, 1977; orig.), antennae ($N = 2$; orig.), wing venation ($N = 1$; Higgins, 1975), eggs and hatching ($N = 3$; Fukuda et al., 1985; Thomas et al., 1991), and life history ($N = 11$; Kurentsov, 1970; Emmet and Heath, 1989; Ebert and Rennwald, 1991; Fiedler, 1991; Thomas et al., 1991; Leimar and Axén, 1993; Korshunov and Gorbunov, 1995; Park and Kim, 1997; Fiedler, 1998; Igarashi and Fukuda, 2000; Beneš et al., 2002; Pierce et al., 2002).

Cladistic methods

We used the maximum parsimony program NONA version 2.0 (Goloboff, 1999), with heuristic search strategy option “hold 1000000; mult*100; hold/100; mult*; max*”, to identify the optimal phylogenetic hypothesis. Tree support was calculated with the bootstrap (NONA: 1000 replications, option “mult*100; hold/100”) and Bremer support (NONA: “bsupport 100000”).

We used parsimony optimization (option “unambiguous” in NONA) to map onto the optimal tree hypothesis the alternative adoption rituals (nos. 84–85 in Appendix 1), larval feeding habits (no. 86), the phylogenetic positions of host plants (no. 80, from Angiosperm Phylogeny Group, 2003), and habitat association (no. 90). The tree topology was tested against the inclusion/exclusion of some characters whose cladistic usefulness seems to be disputable (no. 52, 88, 90).

Results

Phylogeny of Maculinea

The analysis based on the entire data matrix yielded four equally most parsimonious trees (length = 306, consistency index [CI] = 0.33, retention index [RI] = 0.63). In the strict consensus (Fig. 2), *Maculinea* forms a well-supported group, paraphyletic with respect to *Phengaris* (hereafter “*Maculinea* clade”). It is characterized by two unique synapomorphies, both ecological (parasitic myrmecophily, and an obligate association with one ant family, i.e., Myrmicidae). In addition, the group is supported by a short third segment of palps,



Fig. 2. Strict consensus of the four equally most parsimonious trees (length 306, CI 0.33, RI 0.63) showing proposed phylogenetic relationships within the “*Glaucopsyche*-section” of Lycaenidae: Polyommattini. Bootstrap and Bremer support are shown above and below the nodes, respectively.

stalk of forewing veins 7 +8 arising at the end of a discoidal cell, and larva overwintering (Fig. 3). Outside the *Maculinea* clade, *Glaucopsyche* becomes paraphyletic: *G. piasus* belongs to a sister group of *Maculinea*, which includes *Sinia*, *Shijimiaeoides* and *Pseudophilotes*, whereas *G. melanops* and *G. alexis* form a more basal clade with *Caerulea coelestis*.

The *Maculinea* clade is divided into three well-supported subgroups. The basal-most is identical to the “*alcon* group” of Sibatani et al. (1994); it is followed by the “*teleius* group” and the “*arion* group” (including all three species of *Phengaris* in the latter). The *alcon* group shares five synapomorphies, all genitalic, two

being unique to the group (Fig. 3). *M. rebeli* is situated amidst individual “subspecific” taxa of *M. alcon*, as sister to *M. alcon alcon*. The *M. teleius-arion* clade shares five synapomorphies, four of them genitalic and one concerning the position of eggs on the host plant. Two of the genitalic characters are unique to the clade. Within the *teleius* group, *M. kurentzovi* branches off in the basal position; the clade then splits into *M. nausithous* and a monophyletic cluster of putative subspecies of *M. teleius*. The *arion* group is characterized by three genitalic and two wing pattern character states. Within the *arion* group, *M. arionides* seems to be paraphyletic with respect to *Phengaris*.



Fig. 3. One of the four equally most parsimonious trees (length 306, CI 0.33, RI 0.63; chosen at random: individual source trees differ only in position of terminals within *Phengaris*, *M. teleius* and *M. alcon* group: see Fig. 2), with character states that support individual clades. Nonhomoplastic autapomorphies are black, homoplastic apomorphies white. Numbers above branches refer to characters, numbers below branches to character states (see Appendix 1 for character descriptions).

The distribution of apomorphies in the combined tree suggests that terminalia are better at resolving deeper branches than wing patterns, the latter character set being most informative at the specific and subspecific levels (Fig. 3). Only three deeper nodes (*M. nausithous-teleius* clade, *M. arionides-Phengaris* clade, and *Phengaris*) are determined partly or exclusively by the wing-pattern characters. The ecological and life-history characters contribute little to the deep nodes of a tree, with a single but important exception: the monophyly of the *Maculinea* clade is mostly corroborated by the butterflies' ecology.

Evolution of life histories

Parsimony optimization of the presence/absence of the secretion from the dorsal nectary organ (no. 85 in Appendix 1) indicated that use of the DNO secretions during adoption is an ancestral state, which was subsequently lost in the *alcon* group, and either lost or reduced in *M. nausithous*.

Regarding larval feeding (no. 86), we failed to find an unequivocal solution for the origin of predatory and cuckoo habits. The character state is ambiguous in the ancestor of the *Maculinea* clade (Fig. 4A), and it is only certain that the ancestor of the *Maculinea* clade already exhibited obligate parasitic myrmecophily and that the cuckoo and predatory habits evolved in two distinct lineages of the clade. Treating the feeding habit of *M. nausithous* as predatory (or as polymorphic), the optimization of this character on the resulting tree results in three equally most parsimonious solutions:

(i) independent origin of cuckoo and predatory habits, (ii) ancestral predatory habit changed into cuckoo in the *alcon* group, and (iii) ancestral cuckoo habit changed into predatory in the shared ancestor of *arion* and *teleius* groups. Only if *M. nausithous* was recoded as being a cuckoo did two possibilities appear: (i) the origin of predatory habit in the ancestor of the *Maculinea* clade, followed by two changes to the cuckoo habit, in the *alcon* group and in *M. nausithous*; and (ii) the cuckoo habit in the common ancestor followed by independent origins of predatory feeding in *M. teleius* and in the *arion* group. If *M. nausithous* was treated as “intermediate” between predators and cuckoos, this intermediate habit was optimized as ancestral for the *teleius-arion* clade as a whole, followed by two changes to the predatory habit in *M. teleius* and in the *arion* group, while the basal *Maculinea* habit remained equivocal.

The three main groups within the *Maculinea* clade are associated with distinct plant families (no. 80; Fig. 4B): the *alcon* group with Gentianaceae, the *teleius* group with Rosaceae, and the *arion* group with Lamiaceae, with a derived shift to related Gentianaceae and Campanulaceae in *Phengaris daitozana*. These families belong to two distinct clades of angiosperms (Angiosperm Phylogeny Group, 2003), Asteridae (Gentianaceae, Lamiaceae and Campanulaceae) and Rosidae (Rosaceae). The situation at the base of the *Maculinea* clade is ambiguous, but all other deeper-node ancestors within the *Glaucopsyche* section fed on various rosid families.

The common ancestor of the *Maculinea* clade was likely hygrophilous, and two switches to xerophilous

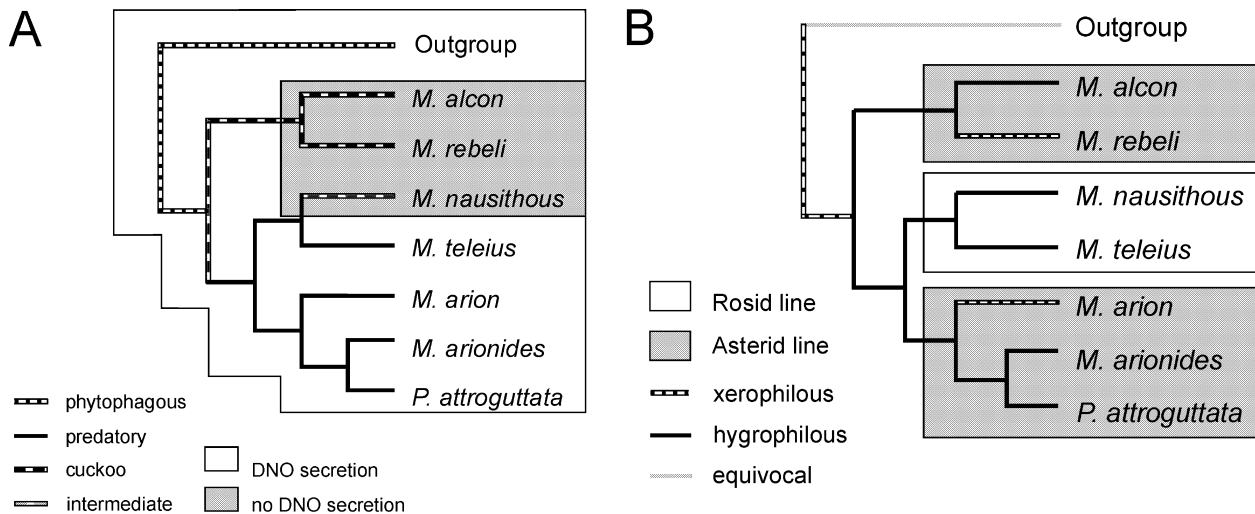


Fig. 4. Evolution of the life history traits of *Maculinea* butterflies and their relatives. Only the species for which states of all relevant characters are reliably well-known are included (see Table 1), but the overall topology of the tree including all terminals (Figs 2 & 3) is preserved. (A) Myrmecophily. *M. nausithous* is optimized as a modified predatory species (see “Evolution of life histories”). (B) Host plants and habitat associations. Rosids and asterids are two well-supported clades of eudicot angiosperm plants (see Angiosperm Phylogeny Group, 2003): Fabaceae and Rosaceae are included in the former, Lamiaceae, Gentianaceae, and Campanulaceae in the latter. In some cases, character-state optimization is derived from the all-species tree (Fig. 3).

habits occurred (no. 90), one in *M. rebeli* and the other in *M. arion*. All deeper-node ancestors within the *Glaucopsyche* section were xerophilous.

Therefore, although it seems certain that the ancestor of the *Maculinea* clade used DNO secretions during the adoption ritual, other arguments for the ancestral predatory habit in the *Maculinea* clade (phylogenetic distribution of feeding habits, assumed sister-group relationship of *Maculinea* and *Phengaris*, Lamiaceae as ancestral host plants, and origin of *Maculinea* in xeric habitats) did not withstand the scrutiny of our cladistic analysis.

Discussion

Phylogeny and classification of Maculinea and Glaucopsyche section

All species of *Maculinea* seem to form a clade that is well differentiated from the other taxa that had been, by different authors, considered congeneric, such as *Glaucopsyche*, *Caerulea* and *Iolana*. The clade splits into three main lineages corresponding, as far as the taxa traditionally classified as *Maculinea* are concerned, to the groupings proposed by Sibatani et al. (1994). All the putatively subspecific taxa of *M. arion* and *M. teleius*, respectively, form monophyletic groups with their nominal forms (Fig. 3). *M. arionides* seems to be paraphyletic, as its putative island subspecies *M. arionides takamukui* from Japan might be a separate species, distinguished by a thick apophysis posterior in the female genitalia and by the absence of spot A3 on the ventral forewing. The West Palaearctic *M.alconalcon* is closer to European *M. rebeli* than to Asian taxa of *M.alcon* s. lat. “*M.alcon*” is then paraphyletic, and *M. rebeli* should possibly lose its full species status. Naturally, the precise relationships among terminal taxa in the present cladogram may be affected by the paucity of character data for some Asian taxa (see Appendix 2). Moreover, the closely related taxa are likely to experience similar selection pressures and to respond to them by parallel adaptation. Some of the relationships between the species (and even more between the subspecific terminals) could easily be due to abundant homoplasies (compare the exceptional non-homoplastic synapomorphies in Fig. 3).

The position of *Phengaris*, which is deeply nested within *Maculinea* in the combined tree, close to “*M. arionides*” and *M. arion*, is quite surprising. Hence, Fiedler’s (1998) “closest living relatives of *Maculinea*” [= *Phengaris*] are, in fact, true *Maculinea* species, which is corroborated by the wing venation and parasitic association with the ants (Fig. 3). It follows that preserving the generic name *Phengaris* renders *Maculinea* paraphyletic, and since *Phengaris* Doherty, 1891 is

an older name than *Maculinea* Van Eecke, 1915, the taxonomy and nomenclature of “*Maculinea*” will need a thorough revision.

However, such formal actions for endangered taxa could seriously affect conservation policy, and we refrain from a formal reclassification of the Polyommata at both genus- (“*Glaucopsyche*”, “*Maculinea*”) and species-rank (“*M. arionides*”, *M. takamukui*?, “*M.alcon*”, *M. rebeli*?) levels until additional data are available. The molecular data will probably add to our understanding of *Maculinea* phylogeny (see <http://www.macman-project.de>; T. Als, D. Nash and K. Pecsénye, pers. comm.), especially in elucidating the terminal relationships within individual lineages. Since morphological and molecular evidence are two autonomous sources of data for reconstructing phylogenies (Freudenstein et al., 2003), the confrontation and combination of different data matrices may corroborate or falsify the validity of the present results (see Vane-Wright, 2003; Wahlberg and Nylin, 2003).

In the same vein, our results regarding the relationships among the *Glaucopsyche* section outgroups must be viewed as only preliminary. The character sampling was affected by the necessity to find traits suitable for finer-level differentiation within *Maculinea*, including characters for putative subspecies, not for an analysis of intergeneric relationships. As a result, many of the traits were not applicable to non-*Maculinea* outgroups (cf. Appendix 2). In addition, the mutual positions of outgroups would likely change by including more non-*Maculinea* terminals and more “genus-level” characters. It still seems likely at this stage that there is no support for the two lineages proposed by Hesselbarth et al. (1995), viz., Scolitantiditi and Glaucopsychiti, and that *Glaucopsyche* is probably paraphyletic and does not represent a single sister group to the *Maculinea* clade.

Origin of the parasitic myrmecophily

The optimization of larval feeding on the tree habits did not support the earlier view by Fiedler (1998), that the predatory habit preceded the cuckoo habit in the evolution of *Maculinea*. However, it did not support any alternative view either, as scenarios of earlier origin of the cuckoo habit, and of two independent origins of the two habits, required identical numbers of evolutionary steps (Fig. 4A). The only firm conclusion is that cuckoo and predatory representatives of *Maculinea* form two distinct clades. The ambiguity with the evolution of cuckoo versus predatory habit might be resolved by an improved understanding of the life history of the little known East Palearctic *M. kurentzovi* (cf. Sibatani et al., 1994; Tuzov et al., 2000) and the problematic *M. nausithous*, basal species of the *teleius* group.

On the other hand, Fiedler (1998) was correct with regard to plesiomorphy of the regular use of DNO

secretions during adoption. Moreover, the finding that *M. nausithous* is not particularly closely related to the *alcon* group suggests that the reduction in the DNO secretions may have evolved twice independently.

The Lamiaceae-feeding predatory taxa of *Maculinea* are derived and not closely related to the more basal Lamiaceae feeders within the *Glaucopsyche* section (e.g., *Pseudophilotes*). The association with Lamiaceae cannot therefore contribute to a resolution of the ancestry of predatory versus cuckoo strategies. The tree topology, and particularly the derived position of *Phengaris*, also refutes the proposal by Fiedler (1998) that ancestors of *Maculinea* evolved in mountain steppe-like habitats with a short vegetation season. The ancestral habitats of *Maculinea* were rather hygrophilous, and the (repeated) colonization of the more dry habitats (*M. rebeli*, *M. arion*) is derived. However, even taxa as closely related as *M. alcon alcon* and *M. rebeli* differ with respect to living at dry versus wet sites. This also seems to be the case in the putatively subspecific taxa of *M. teleius*, whose East-Asian populations reportedly inhabit “dry meadows” (Asahi et al., 1999). The association with humid versus dry habitat conditions thus seems to be rather plastic in *Maculinea*.

Certainly less plastic is the association with host plants, as the three main lineages of the *Maculinea* clade exhibits conservatism on the level of plant families (the highly derived *Phengaris daitozana* is a single exception). On the other hand, remarkable shifts between phylogenetically distant host plants had to occur at the deepest nodes of *Maculinea* phylogeny (Fig. 4B). Equally remarkable is the taxonomic diversity of host plant use by non-parasitic taxa of the *Glaucopsyche* section: *Iolana*, *Glaucopsyche*, *Shijimiaeoides* and *Sinia* feed on Fabaceae; *Scolitantides* and *Philotes* on Crassulaceae; *Pseudophilotes* on Lamiaceae; *Euphilotes* and *Praephilotes* on Polygonaceae; and *Turanana* on Plumbaginaceae. However, each of the non-parasitic genera feed on a single plant family (Fiedler, 1994) and none exhibits the remarkable diversity of the host-plant associations found in *Maculinea*.

Recent studies on nymphalid butterflies have demonstrated that switches towards taxonomically distant plants had been frequent in their evolution, and that their directions depended on plant functional traits, such as chemical composition and plant architecture, rather than on phylogenetic proximity (e.g., Janz et al., 2001; Wahlberg, 2001). It is worth asking why *Maculinea* exhibits such a high diversity in basal nodes combined with strict oligophagy within individual branches. It is plausible that any oviposition-site mistakes, considered as prerequisites for host shifts in herbivorous insects (e.g., Renwick and Chew, 1994; Larsson and Ekblom, 1995), may be more costly in species with an obligate association with ants if the host ants are missing near the erroneously visited host. On the other hand, the

parasitic myrmecophily may occasionally relax the strict dependency on a particular host plant if the proper ant species is present (recall that *Maculinea* larvae are phytophagous for only a short time compared to the permanently phytophagous Lepidoptera). In evolutionary time, this might cause the strict host plant conservatism within individual subclades of *Maculinea*, combined with the radical rearrangement of host plant associations at the base of the whole clade due to rare switches to phylogenetically distant plants. Notably, tropical lycaenids that exhibit mutualistic myrmecophily are often polyphagous with respect to host plants, whereas the Afrotropical genus *Lepidochrysops* Hedicke, 1923, whose representatives employ a *Maculinea*-type parasitic myrmecophily, exhibits oligophagy at the species level while feeding on wide range of plant families (Fiedler, 1995).

The radical rearrangements of host plant association in separate lineages of the *Maculinea* clade also allow for possible repeated emergence of the cuckoo habit in the *M. nausithous* and *M. alcon* groups. We are in agreement with Fiedler (1998), in that it seems reasonable that after a mistaken oviposition, that a predatory species with less developed chemical mimicry and active DNO secretion should be more likely to be adopted than a cuckoo species.

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Appendix 1. List of characters and character states

Male terminalia

0. Genital length from lateral view (0) approximately equal to its height, (1) much wider than its height.

1. Vinculum (0) more or less straight, without a cranially protruding hump, (1) containing prominent cranially protruding hump.

2. Cranial outer angle of socius (0) approximately in the middle between saccus and socius, (1) closer to socius.

3. Hairs on socius in a lateral view (0) form a triangle, (1) form a band, that is slightly broadened on the ventral part of socius, (2) form a band that is not broadened on the ventral part of socius. Unordered.

4. A spike-like protrusion on ventral side of socius, viewed laterally (0) absent, (1) present.

5. Falx (0) long, its base extends the base of socius, (1) short, its base does not reach to the base of socius.

6. Top of ring (0) not protruded, fused with uncus, (1) protruded, extending uncus.

7. Saccus (0) big, (1) very small.

8. Saccus (0) bend caudally, (1) straight or bend cranially.

9. Internal margin of valva, (0) parallel with external margin (1) dilated relative to external margin.

10. Outer margin of valva (0) more or less straight or weakly convex, (1) concave.

11. Ampulla protruding (0) from a middle position at outer margin of valva, (1) from the sharp angle between distal and lateral margins of valva.

12. Base of ampulla (0) wide and rounded externally, (1) narrow and not rounded externally.

13. Base of ampulla (0) smooth, (1) bearing a toothed tubercle.

14. Length of ampulla (0) shorter than 1/2 of distal margin of valva, (1) as long as 1/2 of distal margin of valva or slightly longer, (2) considerably longer than 1/2 of distal margin of valva. Ordered.

15. Outer margin of ampulla (0) bearing teeth, (1) smooth.

16. Teeth on ampulla (0) disarranged, (1) arranged into lines running parallelly with distal margin of ampulla.

17. Length of aedeagus (0) more than height of ring, (1) as long as height of ring or slightly shorter.

18. Length of Chapman's process (0) more than 3/4 of the thorny part of ventral margin of vesica, (1) 3/4–1/4

of the thorny part of ventral margin of vesica, (2) less than 1/4 of the thorny part of ventral margin of vesica. Ordered.

19. Shape of Chapman's process (0) straight, (1) curved.

20. Width of distal part of aedeagus (just behind distal end of Chapman's process) (0) equal or longer than 2/3 of its length, (1) less than 1/2 of its length.

21. Spines on vesica (0) small, (1) large.

22. Laterodistal margin of dorsal sclerite of aedeagus (0) shorter than the width of aedeagus just proximally to vesica, (1) as long as width of aedeagus proximally to vesica, (2) longer than width of aedeagus proximally to vesica. Ordered.

23. Laterodistal margin of dorsal sclerite of aedeagus (0) not dilated, (1) dilated.

24. Laterodistal margin of dorsal sclerite of aedeagus (0) without spines, (1) covered by spines distally.

25. Lateroproximal margin of dorsal sclerite of aedeagus (0) rounded, (1) sharply indented.

26. Distal dilation of long laterodistal process of dorsal sclerite of aedeagus (0) dilates sharply far behind a middle of length of the process, (1) dilates gradually, beginning from a mid-position of the length of the process.

27. Angle between dorsal branches of juxta (0) approximately 50° or less, (1) 60°–70°, (2) 70° or more. Ordered.

28. Inner angle of juxta (0) sharply V-shaped, (1) smoothly U-shaped.

29. An outgrowth inside inner angle of juxta (0) absent, (1) present.

30. Stalk of juxta (0) not dilating laterally, (1) dilating laterally into a bulge-like shape.

Female terminalia

31. Caudal portion of ductus bursae (0) not broadened, (1) broadened.

32. Lodix (0) square, (1) rectangular.

33. Ventral sclerite of VI abdominal segment (0) short and square, (1) oblong and triangular.

34. Genital plate (0) tongue-shaped or triangular, (1) semicircular.

35. Apophysis posterioris (0) relatively thick, (1) relatively slender.

36. Length of papilla analis (0) 0.34–0.39 of VIII tergite, (1) 0.45 of VIII tergite, (2) 0.46–0.54 of VIII tergite. Ordered.

Eggs and hatching

37. Hatching (0) terminal or lateral, (1) basal.

38. Eggs-laying substrates (0) sheltered, (1) exposed.

39. Width of chorion (0) less than 5 µm, (1) more than 5 µm.

Eyes

40. Eyes (0) smooth, (1) hairy.

Palps

41. Length of palps (0) shorter than 1.6 of height of head, (1) longer.

42. Length of third segment of palps (0) short, *c.* 1/4 of length of the second segment, (1) longer, *c.* 1/2 of length of the second segment.

Antennae

43. Ventral side of terminal part of antennae (0) darkly unicoloured, (1) dark with a lightly colored elongate strip.

44. Tip of antennae (0) darkly unicoloured, (1) bearing lightly colored subterminal ring.

Wings, both sexes

45. Stalk of veins 7 + 8 arises (0) at the distal end of discal cell, (1) before distal end of discal cell.

46. Ocellar systems on ventral wings (0) absent, (1) present.

47. Marginal systems on ventral wings (0) absent, (1) present.

Ventral forewing, both sexes

48. Spot A3 (0) absent, (1) present.

49. Spot F3 (0) markedly shifted basally, (1) not markedly shifted basally.

50. Spots B3, C3 and D3 (0) arranged quasi-linearly, (1) in arch-like arrangement.

51. Spots of ocellar system (0) of the same size as spots of other systems, (1) markedly enlarged with respect to spots of other systems.

52. Average forewing length (0) less than 20 mm, (1) more than 20 mm. Coded as ambiguity in species varying around the threshold.

Ventral hindwing, both sexes

53. Spot X (0) absent, (1) present.

54. Spot S (0) absent, (1) present.

55. Spot W (0) absent, (1) present.

56. Spot F3 (0) markedly shifted basally, (1) not markedly shifted basally.

Ventral coloration, both sexes

57. Orange colour between ocellar and marginal systems (0) absent, (1) present.

58. Blue scales on the hind wing (0) absent, (1) present.

59. Basal colour between medial system 3 and ocellar system 2 (0) identical to other parts of the wing, (1) containing white triangles.

60. Fringes on fore wing (0) uniformly coloured, (1) chequered black and white.

61. Fringes on hind wing (0) uniformly coloured, (1) chequered black and white.

62. Basal colour (0) whitish gray, (1) dark gray, (2) dark brown. Unordered.

Ventral forewing, males

63. Spot T (0) absent, (1) present.

64. Spot S (0) absent, (1) present.

65. Spot G3 (0) absent, (1) present.

Ventral hindwing, males

66. Spot T (0) absent, (1) present.

67. Spot V (0) absent, (1) present.

Dorsal forewing, males

68. Spot R (0) absent, (1) present.

69. Spot S (0) absent, (1) present.

Dorsal coloration, males

70. Blue colour (0) extending up to wing margin, (1) surrounded by wide dark marginal strip, (2) substantially reduced. Unordered.

Ventral forewing, females

71. Spot T (0) absent, (1) present.

72. Spot S (0) absent, (1) present.

73. Spot G3 (0) absent, (1) present.

Ventral hindwing, females

74. Spot T (0) absent, (1) present.

Dorsal forewing, females

75. Spot A3 (0) absent, (1) present.

76. Spot S (0) absent, (1) present.

Dorsal hindwing, females

77. Ocellar system on dorsal hind wing (0) absent, (1) present.

Dorsal coloration, females

78. Orange colour on between ocellar and marginal systems (0) absent, (1) present.

79. Blue colour on the dorsal side of wings (0) absent, (1) at least some patches of blue scales, (2) present on more than half of wing surface. Unordered

Ecology and life history

80. Larval host plant (0) Fabaceae, (1) Rosaceae, (2) Lamiaceae, (3) Gentianaceae. Unordered

81. Tentacle organs (0) absent, (1) present.

82. Myrmecophilous habit (0) no stable ant associations, (1) facultative ant mutualism, (2) parasitism. Ordered.

83. Host ant specificity (0) weak, host ants from more families, (1) strong, hosts ants from subfamily Myrmicinae.

84. Adoption (0) fast, without elaborate ritual, (1) slow, with a ritual.

85. Secretion from dorsal nectary organs during adoption (0) absent, (1) always present.

86. Feeding habit of caterpillars within ant nests (0) cuckoo, they are fed by workers via regurgitation, (1) predatory. *M. nausithous* is generally considered predatory (Thomas and Elmes, 1998), but part of this evidence originated from rearing with unnatural ants (Elfferich, 1998). Besides, there are anecdotal reports of larvae being actively fed by ants (mentioned in Elmes et al., 2002), or being tended by them when the nest was disturbed (Stankiewicz and Sielezniew, 2002). We coded the species' habit in four ways: (i) as a "predatory" species, (ii) as a "cuckoo" species, (iii) as an intermediate (0 = predatory, 1 = *M. nausithous*, 2 = cuckoo; ordered); (iv) as a polymorphic ("[01]") trait (Appendix 2; but see Grant and Kluge, 2003; for arguments against polymorphic character coding).

87. During phytophagous stage, larvae feed on (0) vegetative organs or both vegetative and reproductive organs of plants, (1) only on reproductive organs of plants.

88. Voltinism (0) only one generation per year, (1) ability to form more than one generation per year.

89. Overwintering stage (0) caterpillar, (1) pupa.

90. Habitat of the species (0) xerophilous, (1) mesophilous to hygrophilous.

Appendix 2. Data matrix used for phylogeny reconstruction

Lycaena dispar	00--010-0-----0-???0000??-??1?011100110110101111000-0111110-011101110-00-----0101
Polyommatus icarus	111-010-000--11-1-?-?????2100????1?1?1011101101100?10111000111110001111001110110-1-0100
Pseudophilotes vicrama	000001011000-111-1100?????0100????111111101001100100110111110111110010121110-?-?1110
Iolana iolas	00000111000-00010?0???0100?????0100101010001000000101000???001?2001?-?-?010
Shijimiaeoides divina	011?1011010-1[01]011100000-0-1000????1?1??0?111110110110011011011101110110102011?-?-1010
Sinia lanty	01121011111101-010?????1100????1?1??1?111110010011001101111100111?00111?0?0??
Sinia leechi	0002101100111000?0?????2100????1?1??111111100110010111101111002011?00100?0??-?-?0??
Caerulea coelestis	0102011010021-0?????010?????00110011011010100000[12]00101000???00001???-?-?01?
Glaucopsyche piassus	????????111?11-?1?00?????0????????????100010011000?1111[01]1111001?111000020110-?-001?
Glaucopsyche melanops	111100110010-10011?0?0???1100???111111000010000001011011001000010000010110-1-1010
Glaucopsyche alexis	11110110111011-1100110-0100?????0?111110001010000001000100100000010000010110-1-1010
Phengaris atroguttata	01121011100-10001100010-1-1100?????0?0?01101110111110001000101101[01]11111022?21?1100?
Phengaris albida	????????????????????????????????11101110110000110?????101010101??0??21??1???
Phengaris daitozana	?????0?111011?1?110?0???100 0???0??0??011?1110100001100001101023?21?1100?
Maculinea alcon alcon	00120111000-001-1010100-1-21010110111110000101100100010001011100101?0100100000130210001001
Maculinea alcon kondakovi	00?2??1?000-001-1010100-?-???011011??0?0??01100100010001011101101??10110?000?3021??1001
Maculinea alcon arirang	00120111000-001-1010100-0-01000110111??0????01100000?10001011101101??1?1?0000??021??100?
Maculinea rebeli	001201011000-001-1010100-0-21010110111110001101100100?10001011100101?0100100000130210001000
Maculinea arion arion	0010100111011200110101101101000010000001101110100110001011111111[01]1110[01]102202111111000
Maculinea arion xiaheana	00101001110112001101011 0110111000010??0??0?01100100?10001011100011101001??001?021??100?
Maculinea arion ussuriensis	00?????1?101120011010110?????0000010??0????011101011100010111111110111?1001?021??1000
Maculinea arion cyanecula	00?????1?1011120011010110?????0000010??0??1?01110100110001011101111110111??1012021??1000
Maculinea arion inferna	00?????1?1011120011010110?????0000010??0??01110101?1000?011?0??0??101?1?001?021??100?

Maculinea arionides arionides
0002101110111200101010110110000010???000110111011010000101101111111111111022021??11001
Maculinea arionides takamukui
00??01110111001010110??1?0000000?0?0???01100111010001011001111101???0[01]1022021?111100?
Maculinea nausithous
000110111010100020101111100010000200000010000001000000000020010010200100000010210[01][01]1001
Maculinea kurentzovi
00011101010010101011111101100012??0???011101000100000111011011020110000011021???1001
Maculinea teleius teleius
0001101101010102010211??2000100102000001110100100000010000110000110100000000110211111001
Maculinea teleius euphemia
00020011010010102010211002100100102??0?0?1?0110100[01]0101000001001011010110000011021?111001
Maculinea teleius sinalcon
00??01101010102010211102000100102??0?0???01?001000100000012001011020?1???00?021???100?
Maculinea teleius daisensis
00?0??1?0100102010211????100102?0?0???0110000[01]0[01]000?00110110110100100?1011021?11100?
Maculinea teleius hosonoi
00?0??1?010010102010211????100102?0?0???0??1?00??1?0?0?1?0??1?0??20??0001021?11100?
Maculinea teleius kazamoto
00?0??1?0100102010211????100102?0?0???0110000[01]01?00?0011011011010??0000[012]1021?11100?
Maculinea teleius ogumae
00?0??1?010010102010211????100102?0?0???01?0110010001000010111000011010???00[01]001021?11100?
;
cc - 3 62 70 79.80;
cc + 0.2 4.61 63.69 71.78 81.90;