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HOST-PLANT SWITCHES AND THE EVOLUTION OF CHEMICAL DEFENSE AND LIFE HISTORY IN THE LEAF BEETLE GENUS *OREINA*

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Abstract.—Insect-plant interactions have played a prominent role in investigating phylogenetic constraints in the evolution of ecological traits. The patterns of host association among specialized insects have often been described as highly conservative, yet not all specialized herbivorous insect lineages display the same degree of fidelity to their host plants. In this paper, we present an estimate of the evolutionary history of the leaf beetle genus *Oreina*. This genus displays an amazing flexibility in several aspects of its ecology and life history: (1) host plant switches in *Oreina* occurred between plant families or distantly related tribes within families and thereby to more distantly related plants than in several model systems that have contributed to the idea of parallel cladogenesis; (2) all species of the genus are chemically defended, but within the genus a transition between autogenous production of defensive toxins and sequestration of secondary plant compounds has occurred; and (3) reproductive strategies in the genus range from oviparity to viviparity including all intermediates that could allow the gradual evolution of viviparity. Cladistic analysis of 18 allozyme loci found two most parsimonious trees that differ only in the branching of one species. According to this phylogeny estimate, *Oreina* species were originally associated with Asteraceae, with an inclusion of Apiaceae in the diet of one oligophagous species and an independent switch to Apiaceae in a derived clade. The original mode of defense appears to be the autogenous production of cardenolides as previously postulated; the additional sequestration of pyrrolizidine alkaloids could have either originated at the base of the genus or have arisen three times independently in all species that switched to plants containing these compounds. Viviparity apparently evolved twice in the genus, once without matrotrophy, through a retention of the eggs inside the female's oviducts, and once in combination with matrotrophy. We hypothesize that the combination of autogenous defense and a life history that involves mobile externally feeding larvae allowed these beetles to switch host plants more readily than has been reported for highly conservative systems.

Key words.—Allozyme electrophoresis, chemical defense, Chrysomelidae, host plants, *Oreina*, phylogeny, viviparity.

Views about how conservative the host affiliations of herbivorous insects are differ considerably. Beginning with the stimulating review of Ehrlich and Raven (1964) on host use in butterflies, the idea of coevolution between insect lineages and their host plants became popular. More recently, several studies have reported fidelity of insect clades to specific plant clades that could agree with parallel cladogenesis (Zwölfer 1988; Moran 1989; Farrell and Mitter 1990, unpubl. manuscript). If the idea of strict coevolution (which invokes similar ages and mutual selection pressure) is relaxed for less restrictive scenarios, it remains striking that in many insect clades related species feed on related plants and that, overall, insect speciation is rarely associated with shifts to new plant families (Mitter and Farrell 1991). However, host conservatism as a general rule remains questionable (Jermy 1984), and not all specialized herbivores are similarly conservative in their host use.

Phytophagous beetles, one of the largest groups of specialized herbivores, provide convincing examples of potential coevolution (Farrell and Mitter 1990, unpubl. manuscript) or high fidelity to the same host plant tribes (Futuyma and McCafferty 1990; Funk et al. 1995). However, they also provide examples of multiple host shifts among plant families (Brown 1945; Hsiao 1988; Pasteels and Rowell-Rahier 1991; Windsor et al. 1992), which make these beetles a useful group to contrast differing ecological histories and to look for possible underlying causes of host use evolution.

Several life-history or ecological traits are thought to influence the stability of host affiliation. First, species with internally feeding larvae might face more host-imposed constraints than species with externally feeding larvae (Strong et al. 1984). Second, insects that gain protection against predators via host-specific crypsis or host-derived defensive chemicals could be kept on these plants through stabilizing selection from their predators (Bernays and Graham 1988). Limited dispersal abilities or strong adaptations to specific habitats rather than to specific plants, on the other hand, could favor host switches over host tracking (reviewed in Mitter and Farrell 1991).

In this paper, we attempt to reconstruct the evolutionary history of a genus of specialized leaf beetles that displays an amazing flexibility in its ecology and life history. In this genus, *Oreina* Chevrolat, species are found feeding on plants from several tribes of two different plant families, the Apiaceae and the Asteraceae. The host plant influences the beetles' chemical defense, which can either be plant derived and/or autogenously produced. In the former case, pyrrolizidine alkaloid N-oxides are sequestered from Senecioneae (Asteraceae); in the latter case cardenolides are produced from a sterol precursor (e.g., cholesterol) (Pasteels et al. 1994, 1995). Finally, within the genus, an array of different reproductive strategies exists, ranging from oviparity over varying degrees of retention of the eggs to viviparity with nutrition of the embryo inside the female oviducts (Bontems 1989; Dobler and Rowell-Rahier 1996). Although *Oreina* species

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TABLE 1. Collecting sites, main field host, mode of reproduction and defensive compounds of the species used in this study. The code refers to the abbreviation used in some figures and tables. LAM, Lamiaceae; API, Apiaceae; AST, Asteraceae; and PA, pyrrolizidine alkaloids.

Species	Provinience	Code	Host plant	Reproduction	Defensive toxins
C. fastuosa	Blauen, CH	CFaB	*Galeopsis tetrahit,* LAM	oviparous	cardenolides
C. graminis	Pt. Camargue, F	CGrP	*Stachys palustris,* LAM	oviparous	cardenolides
O. bifrons	Hohwald, F	OBiH	*Chaerophyllum hirsutum,* API	viviparous	cardenolides
	Tschiertschen, CH	OBiT	*Chaerophyllum hirsutum,* API	viviparous	cardenolides
O. cacaliae	Tschiertschen, CH	OCaT	*Adenostyles alliariae,* AST	viviparous	PA
	Zastler, D	OCaZ	*Adenostyles alliariae,* AST	viviparous	PA
*O. coerulea**	Bayreuth, D	OCoB	*Centaurea scabiosa,* AST	oviparous	cardenolides
	Calmbach, D	OCoC	*Centaurea nemoralis,* AST	oviparous	cardenolides
O. elongata	Col du Lautaret, F	OElC	*Adenostyles alliariae,* AST	oviparous	PA
	Mattmark, CH	OElM	*Cirsium spinosissimum,* AST	oviparous	cardenolides
O. frigida	Hörnlihütte, CH	OFrH	*Meum athamanticum,* API, *Adenostyles alliariae,* AST	oviparous	cardenolides + PA
O. gloriosa	Saas Grund, CH	OGlS	*Peucedanum osthrutium,* API	viviparous	cardenolides
	Tschiertschen, CH	OGlT	*Peucedanum osthrutium,* API	viviparous	cardenolides
O. intricata	Höllental, D	OInH	*Senecio fuchsii,* AST	viviparous	cardenolides + PA
	Tschiertschen, CH	OInT	*Senecio nemorensis,* AST	viviparous	cardenolides + PA
O. melanocephala	Hörnlihütte, CH	OMeH	*Doronicum clusii,* AST	viviparous	unknown
O. speciosa	Choindez, CH	OSaC	*Chaerophyllum hirsutum,* API	viviparous	cardenolides
	Tschiertschen, CH	OSaT	*Chaerophyllum hirsutum,* API	viviparous	cardenolides
O. speciosissima	Tschiertschen, CH	OSiT	*Petasites albus,* AST	viviparous	cardenolides
	Zastler, D	OSiZ	*Petasites albus,* AST	viviparous	cardenolides
O. variabilis†	Tschiertschen, CH	OVaT	*Chaerophyllum hirsutum,* API	viviparous	cardenolides
	Zastler, D	OVaZ	*Chaerophyllum hirsutum,* API	viviparous	cardenolides
O. virgulata	Hörnlihütte, CH	OViH	*Cirsium spinosissimum,* AST	viviparous	cardenolides

* This species has been addressed as *O. luctuosa* in our previous papers according to Bontems (1984).
† This species is identical to *O. alpestris* according to Kühnelt (1984) and Bourdonné and Doguet (1991).

seem to represent the classic scenario for the gradual evolution of viviparity (see e.g. Blackburn 1992), it remains open whether the observed phenocline actually represents the evolutionary history of this genus.

The role that chemical defense might play in determining diet breadth and host fidelity has been the subject of a recent debate (Bernays and Graham 1988, and responses to this paper). One scenario is that herbivores have specialized on toxic plants under the selective pressure of their natural enemies. Accordingly, the benefit of specialization is increased protection obtained from the host plant, for example, sequestered toxins (Price et al. 1980; Bernays 1988; Bernays and Graham 1988; Hay et al. 1989; Dyer 1995). The question of whether the acquisition of plant-derived defenses precedes or follows autogenous production of defense has been answered differently in two case studies: in leaf beetles, including *Oreina*, it was speculated that host-derived defense is apomorphic to de novo synthesis based on the type of defense observed in related taxa (Pasteels and Rowell-Rahier 1991; Pasteels et al. 1994). This contrasts with the evolution of chemical defense in butterflies, in which autogenous defense is considered to be derived relative to sequestration (Brown et al. 1991).

An independently established phylogeny of the genus *Oreina* will allow to trace the evolution of the beetles ecology and life history. The reconstruction of these traits on a phylogeny of the genus then allows to test the hypotheses outlined above, for example: How often and over which taxonomic distances did the beetles switch host plants? How does this affect defensive chemistry? Does sequestration of plant compounds predate or follow autogenous defenses? In which direction did the life-history strategies evolve? How labile are the life-history and defensive strategies? Here, we establish a phylogeny estimate of the genus through an electrophoretic allozyme study and use this phylogeny to reconstruct the evolution of ecology and life history and to discuss the outlined questions. In our study we consider 12 of 24 species of this largely alpine, palearctic genus (Bourdonné and Doguet 1991), covering the majority of species available in the Alps and all of the ecological variation present in the genus. Most of the omitted species seem to represent locally restricted close relatives or even just forms of these widespread species (Bechyné 1958) and have a similar ecology to those. The two species used as outgroups in this study belong to the closely related genus *Chrysolina* Motschulsky. They share the same number of chromosomes with *Oreina* (Petitpierre and Segarra 1985) and may represent its sister group, the two being occasionally considered as different subgenera of the same genus (Bechyné 1952, 1958; Bourdonnné and Doguet 1991).

Natural History of Oreina

Table 1 gives a summary of the main host plants, the life history, and mode of chemical defense of the beetles under study. In *Oreina*, the mostly aposematic adults and the larvae feed externally on the same plants. Diet breadth varies considerably between the species: among the species feeding on Apiaceae, the food plant spectrum ranges from a single host in *Oreina gloriosa* (*Peucedanum ostruthium*, Peucedaneae), to two hosts of different tribes in *Oreina bifrons* (*Chaerophyllum hirsutum,* Scandiceae and *Peucedanum ostruthium,* Peucedaneae), and to Apiaceae from as many as three different tribes (*Oreina speciosa* and *Oreina variabilis,* feeding

TABLE 2. Relation of females to neonate offspring. The ratio gives the larval/egg weight as percent of female weight. The standard error and the number of individuals are indicated in parenthesis.

Species	Offspring	Weight female (mg)	Weight eggs/larvae (mg)	Ratio
C. graminis	eggs	130.6 (± 10.11; 10)	1.21 (± 0.01; 94)	0.97
O. bifrons	larvae	121.28 (± 3.99; 5)	1.58 (± 0.06; 20)	1.30
O. cacaliae	larvae	96.20 (± 2.85; 10)	0.73 (± 0.03; 20)	0.76
O. coerulea	eggs	150.50 (± 5.66; 10)	1.18 (± 0.05; 20)	0.79
O. elongata	eggs	68.40 (± 2.43; 10)	0.71 (± 0.02; 20)	1.04
O. frigida	eggs	29.30 (± 2.16; 6)	0.27 (± 0.06; 50)	0.92
O. gloriosa	larvae	120.10 (± 4.52; 10)	2.73 (± 0.09; 20)	2.28
O. intricata	larvae	149.85 (± 8.17; 8)	2.45 (± 0.12; 16)	1.63
O. melanocephala	larvae	64.53 (± 2.66; 7)	0.96 (± 0.07; 3)	1.49
O. speciosa	larvae	181.60 (± 8.22; 7)	4.98 (± 0.25; 13)	2.74
O. speciosissima	larvae	63.5 (± 2.36; 9)	0.56 (± 0.03; 16)	0.88
O. variabilis	larvae	154.62 (± 6.92; 10)	4.60 (± 0.27; 20)	2.97
O. virgulata	larvae	67.75 (± 1.50; 6)	0.82 (± 0.06; 6)	1.21

on several Apieae, Peucedaneae and Scandiceae). The majority of *Oreina* species, however, feed on Asteraceae of the tribes Senecioneae and Cardueae. These *Oreina* species all have a narrow diet breadth comprising a single plant genus in some species, that is, *Oreina coerulea* on *Centaurea* species (Cardueae), *Oreina melanocephala* on *Doronicum* species (Senecioneae), and *Oreina virgulata* on *Cirsium* species (Cardueae). Several species feeding on Senecioneae have a slightly broader host range that includes several contribal (*Oreina cacaliae* on *Adenostyles*, *Petasites*, and *Senecio* species, *O. intricata* on *Adenostyles* and *Senecio* species) or intertribal genera (*Oreina elongata* on *Adenostyles* species and *Cirsium spinosissimum* and *Oreina speciosissima* on *Adenostyles*, *Cirsium*, *Doronicum*, *Petasites*, and *Senecio* species). Only a single species, *Oreina frigida*, is known to us that accepts (at least in the laboratory) both Apiaceae and Asteraceae (*Meum athamanticum*, Apieae and *Adenostyles alliariae*, Senecioneae; Pasteels et al. 1995). In several food-choice tests, this species did not show any clear feeding preference between the two plant families.

As previously described, the beetles' defensive chemistry is correlated with their host-plant use: species feeding on Apiaceae or Cardueae produce cardenolides de novo, whereas species feeding on Senecioneae encounter pyrrolizidine alkaloid N-oxides (PAs) in several of their host plants. All species feeding on such plants are able to take up the alkaloids from their hosts and incorporate them into their defensive secretions (Pasteels and Rowell-Rahier 1991; Rowell-Rahier and Pasteels 1992; Pasteels et al. 1995, and unpubl. data for *O. frigida*). In all species except *O. cacaliae*, the sequestration of PAs occurs in addition to the production of cardenolides; *O. cacaliae* relies exclusively on the plant alkaloids. The only species feeding on Senecioneae that does not sequester PAs is *O. melanocephala.* Its food plant, *Doronicum clusii*, does not contain PAs in its leaves, and the beetles reject *D. clusii* leaves treated with PAs (unpubl. results). Moreover, its secretion apparently does not contain cardenolides (Pasteels et al. 1995).

No simple correlation of mode of reproduction with host plant use is obvious: all oviparous species feed on Asteraceae (yet *O. frigida* also takes Apiaceae), but viviparous species are found among Asteraceae and Apiaceae feeders. Among the viviparous species, the amount of matrotrophy the larvae receive inside the female's oviducts varies considerably: the neonate larvae can be up to four times the size (corrected for the mother's size) of larvae hatched from eggs (Table 2).

The two *Chrysolina* species used as outgroups in this study feed on Lamiaceae. Their defensive secretions contain cardenolides (Van Oycke et al. 1988; F. Eggenberger, pers. comm. 1992) and both species lay eggs.

MATERIALS AND METHODS

Insects

All beetles used in the allozyme study were collected during the summer of 1993. The insects were brought to the laboratory alive and were identified and sexed. Males were immediately frozen in liquid nitrogen and kept for later use. Before electrophoresis their genitalia were dissected to confirm species identity. Females of some sympatrically occurring species are difficult to distinguish. For identification, these were kept in the laboratory for up to 3 wk before freezing and allowed to lay larvae. All species can be easily distinguished by larval coloration and morphology. Our nomenclature of the beetles follows Bourdonné and Doguet (1991); the plants are named according to Flora Europaea (Tutin 1980). Collecting sites are indicated in Table 1, a more detailed description is available from the authors upon request.

Electrophoresis

Starch-gel electrophoresis was performed following the procedures and recipes given in Murphy et al. (1990). The thoracic muscles of the beetles were dissected on a chilled plate, divided in two, and one-half immediately homogenized with 50 or 75 μl of cold homogenization buffer (composed of Tris at pH 7, mercaptoethanol, bromephenol-blue, and EDTA). The second half of the muscle preparation was immediately refrozen and kept for reruns of the same individual. Twenty-five individuals were loaded on each gel using 3-mm-wide filter-paper wicks. To aid comparisons among gels, three individuals of the Saas Grund population of *O. gloriosa* were loaded on one side of each gel and each of these three individuals loaded a second time inside the same gel to provide an internal standard. To optimize resolution of alleles, the

TABLE 3. Genetic distances between the Oreina and Chrysolina populations. Above diagonal Nei's unbiased genetic distance (*** not defined), below diagonal Cavalli-Sforza and Edward's chord distance. Codes for populations as in Table 1.

	CFaB	CGrP	OBiH	OBiT	OCaT	OCaZ	OCoB	OCoC	OElC	OElM
CFaB	****	1.505	2.559	2.084	2.343	2.153	1.612	1.942	2.822	2.747
CGrP	0.834	****	2.816	2.384	1.856	1.814	1.472	1.539	3.472	2.363
OBiH	0.926	0.936	****	0.075	1.839	2.025	1.573	1.717	2.768	2.17
OBiT	0.877	0.894	0.267	****	1.721	1.898	1.347	1.46	1.955	1.579
OCaT	0.876	0.848	0.864	0.829	****	0.03	1.361	0.966	1.052	0.736
OCaZ	0.877	0.855	0.889	0.854	0.19	****	1.363	0.987	1.152	0.889
OCoB	0.822	0.808	0.836	0.784	0.772	0.783	****	0.131	1.807	1.27
OCoC	0.882	0.846	0.881	0.829	0.735	0.748	0.337	****	1.814	1.14
OElC	0.915	0.931	0.933	0.868	0.745	0.773	0.839	0.872	****	0.235
OElM	0.936	0.922	0.929	0.856	0.686	0.736	0.8	0.806	0.446	****
OFrH	0.817	0.904	0.926	0.875	0.789	0.824	0.878	0.89	0.767	0.729
OGlS	0.944	0.95	0.85	0.813	0.817	0.849	0.891	0.928	0.893	0.888
OGlT	0.95	0.955	0.852	0.815	0.821	0.853	0.897	0.933	0.892	0.888
OInH	0.869	0.894	0.813	0.768	0.778	0.806	0.748	0.813	0.79	0.766
OInT	0.871	0.894	0.804	0.766	0.778	0.806	0.749	0.817	0.798	0.782
OMeH	0.889	0.934	0.926	0.914	0.813	0.844	0.877	0.92	0.903	0.928
OSaC	0.907	0.955	0.839	0.827	0.809	0.861	0.925	0.963	0.89	0.891
OSaT	0.907	0.942	0.809	0.798	0.799	0.85	0.909	0.95	0.876	0.881
OSiT	0.814	0.841	0.893	0.839	0.598	0.602	0.787	0.806	0.697	0.693
OSiZ	0.811	0.802	0.878	0.829	0.539	0.555	0.762	0.762	0.696	0.631
OVaT	0.872	0.904	0.859	0.826	0.794	0.853	0.882	0.912	0.847	0.817
OVaZ	0.883	0.905	0.865	0.845	0.803	0.855	0.882	0.913	0.865	0.85
OViH	0.848	0.935	0.982	0.933	0.798	0.804	0.904	0.92	0.809	0.823

gels were run out until the tracking dye reached the anode; then they were cut in three to six slices and stained. In a preliminary study, 31 enzyme stains were tested on six different buffer systems. Of these, 16 enzymes were scorable, yielding a total of 18 presumptive loci. Best separation was obtained using three different buffer systems. Aconitase Hydratase (ACOH, EC 4.2.1.3 [two loci]), Adenylate Kinase (AK, EC 2.7.4.3), Arginine Kinase (ARK, EC 2.7.3.3), Dihydrolipoamide Dehydrogenase (DDH, EC 1.8.1.4 [two loci, only one scorable]), Glucose-6-phosphate Isomerase (GPI, EC 5.3.1.9), Malate Dehydrogenase (MDH, EC 1.1.1.37 [two loci, only one scorable]), Phosphogluconate Dehydrogenase (PGDH, EC 1.1.1.44), and Phosphoglucomutase (PGM, EC 5.4.2.2) were resolved on a discontinuous tris-citrate buffer pH 7.1 (Ayala et al. 1972). Aspartate Aminotransferase (AAT, EC 2.6.1.1 [two loci, only one scorable]), Formaldehyde Dehydrogenase (FDH, EC 1.2.1.1) and Triose-phosphate Isomerase (TPI, EC 5.3.1.1 [recipe from Werth 1985]) were resolved on a discontinuous tris-borate EDTA buffer pH 8.6 (Murphy et al. 1990). Isocitrate Dehydrogenase (IDH, EC 1.1.1.42 [two loci]), Malate Dehydrogenase-NADP$^+$ (MDHP, EC 1.1.1.40) and three different Peptidases (PEP-B, EC 3.4.11.4; PEP-D, EC 3.4.13.9 [two loci, one scorable]; a dipeptidase using L-leucyl-DL-alanine as substrate, EC 3.4.13.11 (three loci, one identical to PEP-B, one not scorable) were resolved on a tris-citrate buffer pH 8.0. After staining, all gels were first analyzed by measuring migration distances. In addition, digitized pictures of each gel were taken with a video camera connected via a video interface to a Macintosh computer. These pictures were later used for final analysis with the National Institutes of Health (NIH) software IMAGE. Identity of electromorphs was established by side-by-side comparison on the same gel. The alleles were assigned letters in order of decreasing mobility towards the anode. Isozyme loci are numbered from cathode to anode.

Data Analysis

Two methods of phylogenetic inference were used to analyze the electrophoretic data: one based on genetic distances derived from gene frequencies and the second based on cladistic analysis of discrete characters (individual allozyme loci). Gene frequencies and genetic distance coefficients were computed using BIOSYS release 1.7 (Swofford and Selander 1981). BIOSYS was also used to perform distance Wagner analysis using Cavalli-Sforza and Edwards chord distances with outgroup rooting of the tree. The addition sequence of taxa in the Wagner tree was determined by the multiple addition criterion algorithm of Swofford (1981). Additional analysis based on genetic distances used the Fitch-Margoliash method ($P = 2$) with the Cavalli-Sforza and Edwards chord distance (computed by PHYLIP) and a bootstrap analysis (100 samples) to get confidence levels on the nodes.

Cladistic analysis was done by coding the locus as the character with all existing allele combinations as character states. Following Mabee and Humphries (1993), we used step matrices to assign costs to all possible character-state changes for each locus with equal weighting of gains and losses of alleles. This approach allows use of the locus as the character, which has been suggested as the appropriate coding procedure for allozyme data (Mickevich and Mitter 1983; Buth 1984; Murphy 1993), while including at the same time all available homology information. The analysis was performed using the stepmatrix option in PAUP and the general heuristic search algorithm (Swofford 1993). As an additional refinement of the procedure, we ran a second analysis that includes reconstructed ancestral states in the stepmatrices following the procedure of Mardulyn and Pasteels (1994). The bootstrap procedure was used to get confidence levels on the most parsimonious trees resulting from both analyses. In addition, we performed 100 replications of the heuristic search with

TABLE 3. Extended.

OFrH	OGlS	OGlT	OInH	OInT	OMeH	OSaC	OSaT	OSiT	OSiZ	OVaT	OVaZ	OViH
1.272	4.794	4.806	1.87	1.907	1.924	2.338	2.644	1.659	1.772	2.057	2.214	1.486
2.116	****	****	2.23	2.244	2.812	****	****	1.985	1.647	2.749	2.699	2.814
2.199	1.474	1.448	1.234	1.183	2.194	1.349	1.228	2.714	2.65	1.664	1.706	6.255
1.821	1.372	1.344	1.106	1.096	2.482	1.434	1.31	2.092	2.16	1.584	1.769	3.027
1.21	1.498	1.489	1.231	1.239	1.365	1.395	1.409	0.605	0.468	1.425	1.485	1.257
1.381	1.71	1.696	1.361	1.362	1.543	1.785	1.8	0.596	0.491	1.928	1.936	1.242
2.118	2.717	2.729	1.077	1.086	2.095	5.184	4.527	1.647	1.521	2.924	2.865	2.668
1.838	2.8	2.811	1.293	1.322	2.278	****	****	1.499	1.252	2.765	2.715	2.26
1.004	2.181	2.075	1.193	1.248	2.125	2.036	2.018	0.892	0.928	1.735	1.937	1.218
0.791	1.81	1.743	0.98	1.057	2.176	1.773	1.8	0.801	0.621	1.299	1.539	1.189
****	1.767	1.702	0.973	1.055	2.082	1.674	1.834	0.797	0.782	1.249	1.342	1.255
0.88	****	0.005	2.187	2.296	1.545	1.231	1.202	1.439	1.616	1.101	1.155	1.551
0.879	0.081	****	2.081	2.177	1.499	1.221	1.192	1.465	1.615	1.099	1.151	1.549
0.761	0.894	0.894	****	0	2.41	1.847	1.992	1.194	1.246	1.608	1.667	1.801
0.778	0.899	0.899	0.061	****	2.363	1.798	1.916	1.295	1.342	1.677	1.699	1.895
0.917	0.857	0.857	0.919	0.916	****	1.037	1.019	2.968	2.224	1.11	1.087	1.799
0.876	0.807	0.81	0.877	0.872	0.782	****	0.025	1.489	1.546	0.174	0.168	2.832
0.879	0.791	0.794	0.875	0.869	0.768	0.17	****	1.628	1.665	0.204	0.2	2.803
0.688	0.794	0.803	0.757	0.772	0.898	0.806	0.808	****	0.096	1.149	1.277	0.933
0.676	0.801	0.807	0.754	0.767	0.858	0.8	0.799	0.281	****	1.17	1.296	0.878
0.805	0.767	0.771	0.836	0.842	0.78	0.384	0.406	0.739	0.731	****	0.028	2.358
0.82	0.778	0.782	0.844	0.846	0.777	0.379	0.403	0.761	0.752	0.173	****	2.59
0.83	0.859	0.864	0.881	0.888	0.896	0.944	0.931	0.723	0.701	0.907	0.918	****

random addition sequences to evaluate the efficiency of the analysis described above in finding the shortest tree for our data. Tracing of character evolution and presentation of the trees from the cladistic analysis was done with MacClade (Maddison and Maddison 1992).

RESULTS

Genetic distance analyses of the data yielded different phenograms depending on the distance measure used (Cavalli-Sforza and Edwards chord distances and Nei's unbiased genetic distances are given in Table 3; a table with allele frequencies for all loci can be obtained from the authors upon request). The distance Wagner tree and the Fitch-Margoliash tree with bootstrap values (both based on Cavalli-Sforza and Edwards chord distances) are presented in Figures 1 and 2. The phenograms agree on the *Oreina* species being a monophyletic sister group to the two *Chrysolina* species we used as outgroups in this study. The distances to both outgroups are large (0.8–0.9), but not much larger than between some taxa of *Oreina* (e.g., between *O. coerulea* or *O. intricata* and all others). In all phenograms, populations of the same species are grouped together, *O. coerulea* has a basal position and two major clusters within *Oreina* appear: *O. cacaliae*, *O. elongata*, and *O. speciosissima*, although in slightly different branching patterns, and *O. bifrons, O. gloriosa, O. speciosa*, and *O. variabilis*.

The parsimony analysis of the data with the locus coded as the character and all possible transformation series of allele combinations coded by step matrices resulted in two most parsimonious trees of a total length of 361 steps. Only two trees resulted that were one step longer. Additional inclusion of hypothetical ancestral states as proposed by Mardulyn and Pasteels (1994) gave the same results, the two maximum-parsimony trees being only slightly shorter (357 steps). In both trees and in accordance with the genetic distance analyses, the *Oreina* species figure as monophyletic to the two outgroup *Chrysolina* species. *Oreina frigida* is the basal lineage of *Oreina* followed by a clade of *O. coerulea*, *O. elongata*, *O. speciosissima*, and *O. cacaliae*, to which we will further refer as the cacaliae clade. A second major group consists of *O. gloriosa*, *O. bifrons*, *O. variabilis*, and *O. speciosa*, which we will call the gloriosa clade. *Oreina virgulata* and *O. melanocephala* branch at the bottom of this clade. The single topological difference between the two most parsimonious trees is the position of *O. intricata*, which is positioned in both trees between the two major groups, the disagreement being whether it branches at the bottom of the cacaliae clade or right before *O. melanocephala* at the bottom of the gloriosa clade. Figure 3 presents a consensus of the two trees.

As character ordering by step matrices and the high number of taxa included greatly increase computation times, analysis of our data with the branch-and-bound algorithm in PAUP was not possible. To test the efficiency of the heuristic search algorithm to find the shortest trees for our data, we therefore ran 100 replications of the search with random addition sequence of the data. The resulting trees corresponded in 58 cases to the most parsimonious trees described above, in 41 cases the search came up with three trees one step longer, and in one case only the result was a single tree of a length of 366 steps. This highly consistent result strongly suggests that the search algorithm actually found the most parsimonious trees, and thus that further tree families, which might include shorter trees, do not exist.

Because both analysis methods (phenetic and cladistic) group the two populations of each species (bootstrap values in the Fitch-Margoliash tree of mostly 100% [96% being the lowest]), we pooled these populations in one OTU combining

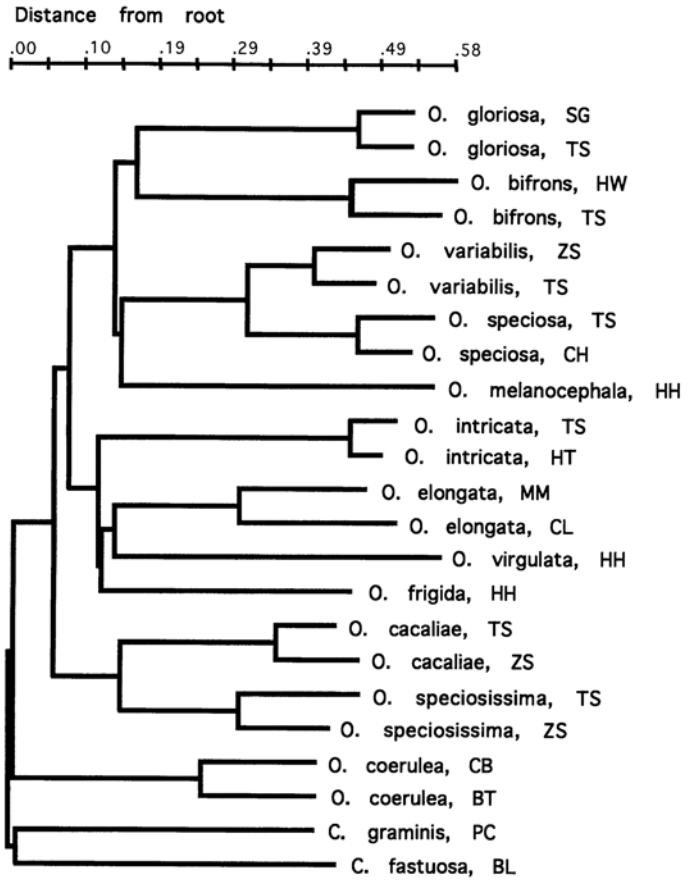

FIG. 1. Distance Wagner tree with outgroup rooting based on Cavalli-Sforza and Edwards chord distances. Multiple addition criterion procedure of Swofford (1981) was used to determine addition sequence retaining five networks at each step and using Prager and Wilson's *F*-value to select partial networks. Total length of tree = 6.331, Prager and Wilson (1976) $F = 6.728$, percent standard deviation (Fitch and Margoliash 1967) = 9.462, cophenetic correlation = 0.941. Abbreviations for locations: BL, Blauen; BT, Bayreuth; CB, Calmbach; CH, Choindez; CL, Col du Lautaret; HH, Hörnlihütte; HT, Höllental; HW, Hohwald; MM, Mattmark; PC, Petite Camargue Alsacienne; SG, Saas Grund; TS, Tschiertschen; and ZS, Zastler.

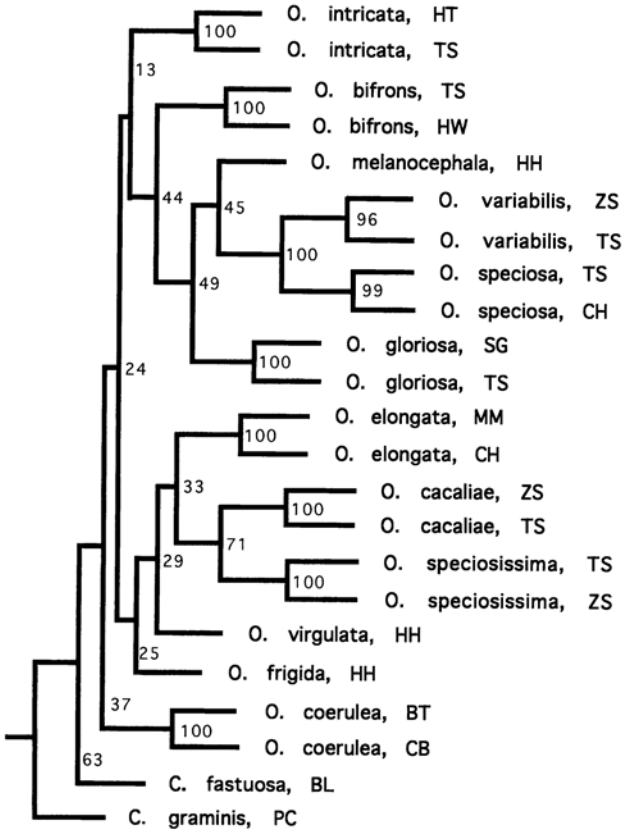

FIG. 2. Fitch-Margoliash tree based on Cavalli-Sforza and Edwards chord distance. Numbers on branches are bootstrap values from 100 samples. Abbreviations for locations as in Figure 1.

all alleles present in each species. We then calculated confidence levels for the maximum parsimony trees by running 500 bootstrap samples on this reduced data set (Fig. 4).

DISCUSSION

Choice of Analysis Methods

Allozyme data have the great advantage of combining easily accessible information from several genes, which in general are probably not under similar selection pressures. However, the optimal analysis of allozyme data has been controversial. Although phenetic analyses of genetic distances or cladistic analyses treating the individual allele as a character have predominantly been used, cladistic analysis recognizing the locus as the character has been advanced as the most appropriate analysis method, due to nonindependence of alleles at a locus (e.g., Mickevich and Mitter 1983; Buth 1984; Swofford and Olsen 1990; Murphy 1993). However, the lack of a satisfactory algorithm for ordering the character states (i.e., the observed allele combinations at each locus) has limited the application of this method, especially in cases in which the species are highly polymorphic. The procedure proposed by Mabee and Humphries (1993) seems promising: the enzyme loci are coded as the phylogenetically informative characters, at the same time all observed allele combinations are included as character states, and all possible transitions between the allelic combinations at a locus are ordered by stepmatrices. Finally, the data are subjected to parsimony analysis. We consider this method to be the best for analyzing our allozyme electrophoretic data, which show high amounts of fixed allelic differences and high levels of polymorphism for some loci. For comparison, we also present the outcome of two methods based on genetic distances, distance Wagner analysis and the least-squares method of Fitch and Margoliash.

Conflicts between the Topology of the Distance and Parsimony Trees

The topology obtained with both methods of analysis is reasonably similar: (A) *O. cacaliae*, and *O. speciosissima* are close relatives with *O. elongata* clustering close to them; (B) *O. melanocephala* plus *O. gloriosa*, *O. bifrons*, *O. variabilis*, and *O. speciosa* form a group; and (C) *O. intricata* stands between these two clusters.

The position of three species differs between the two types

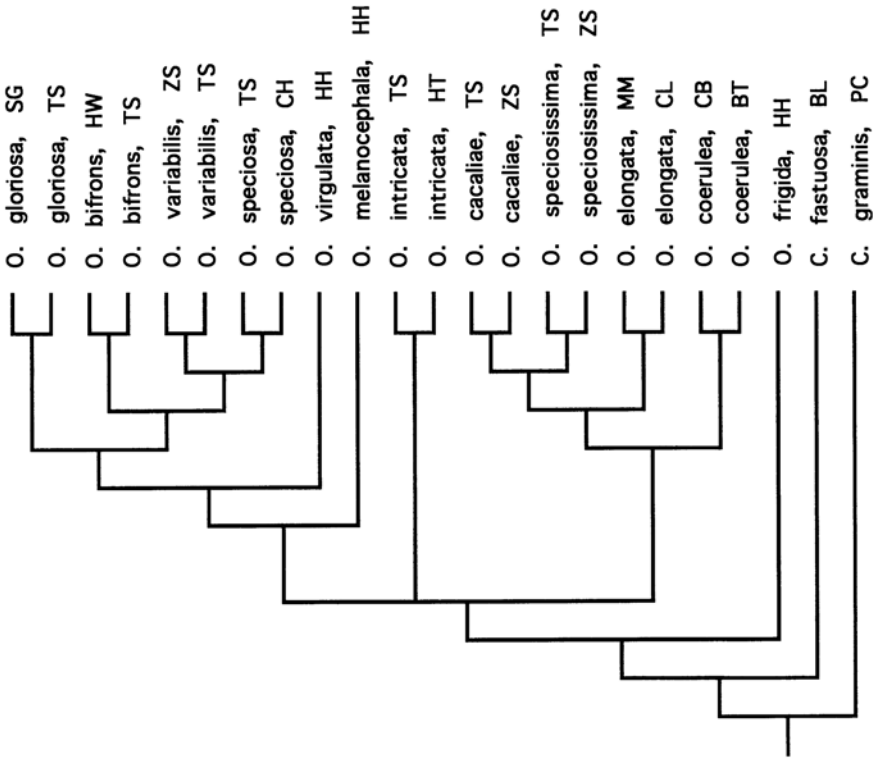

FIG. 3. Strict consensus of two maximum-parsimony trees obtained by ordering the allelic combinations through stepmatrices. The same two trees result whether or not additional ancestral states are included in the computation. Abbreviations for locations as in Figure 1.

of trees: (a) *O. coerulea*, which is placed in a basal position in both types of analyses, is clearly different from all other *Oreina* in the distance analyses yet groups with the cacaliae clade in the parsimony analysis; (b) *O. frigida* clusters with *O. elongata* and *O. virgulata* in the distance analyses but is the most basal clade in the parsimony analysis; and (c) *O. virgulata* appears close to the gloriosa clade in the parsimony analysis but groups with *O. elongata* and *O. frigida* in the phenograms.

The bootstrap values for the position of these species in the Fitch-Margoliash tree as well as the maximum-parsimony trees are below the originally proposed bootstrap value of 95% (Felsenstein 1985) and the reassessed 70% bootstrap value for a 95% confidence interval of an actually correct

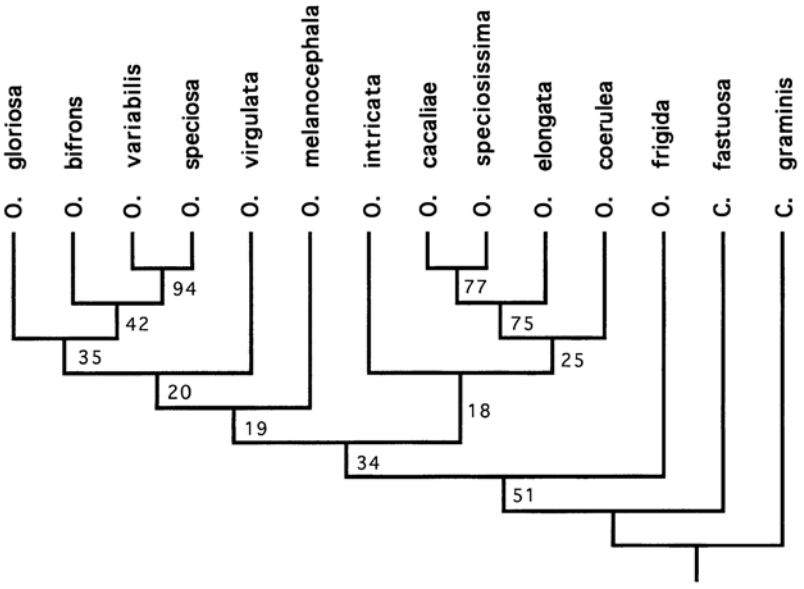

FIG. 4. Maximum-parsimony tree with bootstrap values from 500 samples. For each species with two populations, these have been pooled into one sample.

position of the species (Hillis and Bull 1993). All three species branch with short internodes in the distance trees, which also indicates that due to the high amount of fixed allelic differences unequivocal information for their placement is lacking. According to the theoretical considerations on the choice of analysis method, we will base our discussion of the evolution of ecological characteristics on the consensus tree obtained with parsimony analysis. We will discuss only an alternative placement of the species when this drastically alters the reconstruction of the ecological traits.

Comparison of the Maximum-Parsimony Tree with Conventional Classification Based on Morphology

The genus *Chrysolina* and *Oreina* are often considered a single genus (Bechyné 1952, 1958; Bourdonné and Doguet 1991). In our phylogeny estimate, *Oreina* clearly appears monophyletic relative to the two *Chrysolina* species used as outgroups. A comparison of our phylogeny estimate with the classical subgenus division of *Oreina* (e.g. Bontems 1978) shows the following. (A) The subgenus *Oreina s.str.* (*Oreina basilea*, *O. bifrons*, *Oreina ganglbaueri*, *Oreina gloriosa*, *Oreina liturata*, *O. speciosa*, *O. variabilis* [= *Oreina alpestris*], *Oreina viridis*) is monophyletic in the maximum parsimony analysis. *Oreina intricata* was originally placed in this subgenus but strongly differs in its ecological characteristics and has been placed in its own subgenus on the grounds of genital morphology by Kühnelt (1984). The separation of *O. intricata* from the *Oreina s.str.* is supported by our results as it appears both in the parsimony and genetic distance analyses. (B) The original subgenus *Chrysochloa* (*O. cacaliae*, *Oreina elegans*, *O. elongata*, *Oreina fairmairiana*, *O. frigida*, *O. speciosissima*, and *O. virgulata*) is split into three different clades in our phylogeny: one comprises *O. cacaliae*, *O. elongata*, and *O. speciosissima*, the next *O. virgulata*, and the third *O. frigida*. Thus, this subgenus does not seem to be monophyletic. These results correspond with Kühnelt's (1984) morphology-based revision: he claimed that *O. frigida* and *O. virgulata* are misplaced inside this subgenus and assigned both to new subgenera of their own. (C) The subgenus *Protorina* (*Oreina ludovicae*, *O. melanocephala*, *Oreina plagiata*, *Oreina retenta*, *Oreina sybilla*) represented here by *O. melanocephala* is nested between two of the newly defined groups, *O. virgulata* and *O. intricata*. (D) The subgenus *Allorina* (*Oreina bidentata*, *Oreina canavesei*, *O. coerulea*, and *Oreina collucens*) represented by *O. coerulea* appears at the bottom of the remaining *Chrysochloa* and next to *O. intricata*.

Thus, the groups defined by our phylogeny are concordant with the morphological classification as it emerged from the last thorough revision (Kühnelt 1984). A cladistic analysis of the morphological data has never been attempted and seems unpromising given the excessive variability between populations and the small number of useful synapomorphies (e.g., Bechyné 1958; Kühnelt 1984).

Evolution of Host-Plant Association

We first want to reconstruct the evolution of affiliations with plant families on our phylogeny estimate (Fig. 5). An ancestral association with both the Asteraceae and the Apiaceae or with only the Asteraceae are equally parsimonious possibilities (i.e., require the minimum number of host shifts). Only three events (counting losses and gains separately) are necessary to reach the present state. In the case of both plant taxa being ancestral these are a loss of Apiaceae feeding on branch (A) and simultaneous loss of Asteraceae feeding and regain of Apiaceae-feeding on branch (B). If Asteraceae feeding is considered ancestral, there must be a gain of Apiaceae feeding on the branch leading to *O. frigida* and a loss of Asteraceae feeding combined with an independent parallel gain of Apiaceae feeding at the bottom of the gloriosa clade (B). With a position of *O. frigida* in the middle of the Asteraceae feeding species as suggested by the phenograms an ancestral association with Asteraceae and two independent switches to Apiaceae would be most likely; on the other hand, a position of *O. melanocephala* within the Apiaceae-feeding gloriosa clade as indicated in the genetic distance analysis would require a complete reversal to Asteraceae feeding in this species adding two more steps to host evolution.

Chrysolina, the sister genus of *Oreina*, comprises a vast array of subgenera specialized on different plant families of Dicotyledones. Although the majority of species are found on Lamiaceae and Scrophulariaceae, both Apiaceae and Asteraceae (among other plant families) are also used by some subgenera (Bourdonné and Doguet 1991). However, a reliable phylogeny of *Chrysolina* has not yet been established and the position of these subgenera relative to *Oreina* and the other *Chrysolina* remains unclear.

Below the plant family level, no clear pattern of host-plant switches appears in *Oreina*. Species on Asteraceae are either associated with Senecioneae only (*O. cacaliae*, *O. frigida*, *O. intricata*, and *O. melanocephala*), with Cardueae only (*O. coerulea*, and *O. virgulata*) or accept plants from both tribes (*O. elongata*, and *O. speciosissima*). Beetle species affiliated with one or the other tribe do not form groups on our cladogram nor would an alternative position of *O. frigida* or *O. virgulata* as suggested by the phenograms sort beetles by host-plant tribes. We must therefore postulate multiple switches between these tribes. These two asteraceous tribes are phylogenetically distant within the Asteraceae (Bremer 1994) and comprise chemically distinct lines that differ through the presence of pyrrolizidine alkaloids and a unique type of sesquiterpene-lactone in the Senecioneae and the absence of acetylenes in this tribe which are for the rest typical for both Asteraceae and Apiaceae (Hegnauer 1977; Mabry and Bohlman 1977). At least for the pyrrolizidine alkaloids, we can experimentally demonstrate that they do constitute a chemical feeding barrier for beetles not adapted to them: several species (*O. coerulea*, *O. bifrons*, and *O. melanocephala*) rejected leaves of their host plants if those were coated with PA-N-oxides. Apiaceae used as hosts by *Oreina* species belong to three different tribes, that is, Apieae (*Meum*), Peucedaneae (*Angelica*, *Heracleum*, and *Peucedanum*), and Scandiceae (*Chaerophyllum*, and *Anthriscus*). In the gloriosa clade (B) *Peucedanum ostruthium* (Peucedaneae) is the only plant (tribe) that all these species feed on readily; thus, in this clade, either a successive broadening of the food spectrum might be hypothesized or a secondary restriction to monophagy in *O. gloriosa*. *Oreina frigida*, the Apiaceae-feeding species outside the gloriosa clade, is specialized on

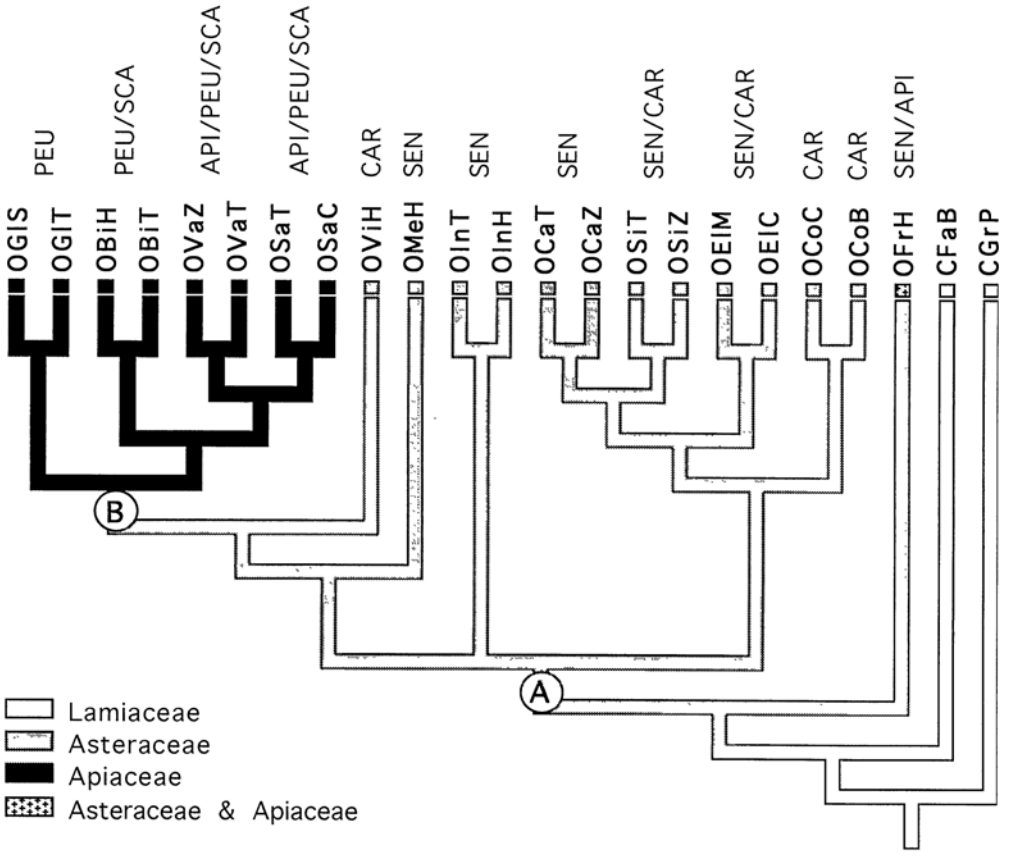

FIG. 5. Reconstruction of host-plant affiliation in *Oreina* based on the consensus of the two maximum-parsimony trees. For each species, the food plant tribes are given: API, Apieae; CAR, Cardueae; PEU, Peucedaneae; SCA, Scandiceae; and SEN, Senecioneae. Beetle species and locations coded as in Table 1. A and B mark branches discussed in detail in the text.

a different tribe (Apieae). Despite high levels of secondary compounds in the Apiaceae in general, the chemical differences between these tribes seem to be lower than between the tribes of Asteraceae in question, that is, not defined by presence or absence of compound classes but rather by differences in their chemical structure (Hegnauer 1971).

It is interesting to note that in *Oreina*, host fidelity is already lost below the plant family level, and no pattern of evolutionary association exists at the tribal level or below. This contrasts with several other studies on herbivorous beetles: in the genus *Ophraella*, all species feed on asteraceous plants and host affiliations are remarkably conservative at the tribal level. However, the number of inferred switches increases drastically at the level of plant genus and involves many parallelisms (Futuyma and McCafferty 1990; Funk et al. 1995). Two beetle genera studied, *Phyllobrotica* and *Tetraopes*, showed even more conserved host associations, hinting towards strict coevolution between the beetles and their host plants (Farrell and Mitter 1990, unpubl. manuscript). Evidence why *Oreina* and also the sister genus *Chrysolina* are more flexible in their host affiliations is still missing, yet it is tempting to speculate that the combination of autogenously produced defensive chemistry and aposematism frees the beetles of possible constraints imposed by host plant-dependent crypsis or dependence on defensive plant compounds (as is possibly the case in *Phyllobrotica* and certainly in *Tetraopes*, B. Farrell and S. Malcolm pers. comm.). Additionally, a trend for higher host flexibility in genera with leaf-feeding larvae (*Chrysolina, Ophraella*, and *Oreina*) compared to genera with root-feeding larvae (*Phyllobrotica* and *Tetraopes*) seems obvious. This accords well with theories on stronger host-imposed constraints for internal feeders (Strong et al. 1984). Other leaf beetle genera in which many host switches occurred also combine externally feeding larvae with autogenous defense (e.g., *Calligrapha* Brown 1945; Pasteels 1993; and *Gonioctena* Mardulyn and Pasteels, unpubl. manuscript).

At least some *Oreina* species might switch to available hosts within a given restrictive habitat before they track hosts across habitats. In the high-altitude environment in which many *Oreina* species live, host-plant availability might actually be the decisive limiting factor. *Oreina frigida*, for example, is found under stones at the alpine vegetation line—the incorporation of plants from two different families in its diet could simply mirror the available choice and plant abundance. This kind of "opportunistic shift" has been considered a common phenomenon in chemically defended leaf beetles (Pasteels and Rowell-Rahier 1991). Cases of habitat fidelity rather than host fidelity have also been described for donaciine leaf beetles, pyralid moths, and various weevils (reviewed in Mitter and Farrell 1991). In tropical butterflies that could establish themselves in temperate habitats, host shifts

might similarly have been dictated by lack of plants related to their original hosts (Scriber et al. 1991).

In general, we favor ecological explanations for host switches in *Oreina*. However, a detailed study of *Ophraella* leaf beetles pointed out that biases in the existing genetic variation might explain realized host shifts and their occurrence mostly among related chemically similar plants (Futuyma et al. 1993, 1995). Even if genetic variation in host use is generally rare, *Oreina* provides an example that shifts can occur to more distantly related and chemically dissimilar plants in which feeding barriers have to be overcome.

Evolution of Defensive Chemistry

In the 12 species of *Oreina* under scrutiny, six rely exclusively on cardenolides for their defense, four combine in their secretions autogenous cardenolides with sequestered pyrrolizidine alkaloids (with variation between populations), and only one (*O. cacaliae*) relies exclusively on sequestered PAs. In the following discussion, we will ignore *O. melanocephala*, as we are still uncertain about the composition of its defensive secretions. However, we know that *O. melanocephala* does not encounter pyrrolizidine alkaloids in its host and is repelled if leaves of its food plant are treated with PAs (Pasteels et al. 1995, and unpubl. results).

The distribution of chemical defense characteristics on our cladogram allows three equally parsimonious reconstructions for their evolution. All of these scenarios agree that the production of cardenolides is the ancestral condition shared with the outgroups (and many other species of the Chrysolinina), as previously postulated (Pasteels and Rowell-Rahier 1991; Pasteels 1993; Pasteels et al. 1994). All three scenarios require four steps to explain the gains and/or losses of the ability to sequester PAs and the loss of cardenolide production in *O. cacaliae* (Fig. 6). If reversals are favored over parallelisms, then both possible positions of *O. intricata* require one gain of PA sequestration in the ancestor of *Oreina* followed by a loss of this capacity in *O. coerulea* and a second loss after the branching of *O. intricata* (Fig. 6A). If, on the other hand, parallelisms are favored over reversals, three independent origins of sequestration are postulated, regardless of the position of *O. intricata* (Fig. 6B). The third possible scenario requires a position of *O. intricata* at the base of the cacaliae clade and involves one origin of sequestration in the branch leading to *O. frigida* and a second in the cacaliae clade (including *O. intricata*), combined with a loss of sequestration in *O. coerulea* (Fig. 6C). This latter scenario seems less likely than the two preceding (see section on life history).

Several origins of sequestration of pyrrolizidine alkaloids might appear unlikely, but, for example, larvae of *Phratora* and *Chrysomela* species have independently adopted the sequestration of the same plant compounds following identical host-plant switches (Pasteels and Rowell-Rahier 1991; Hsiao et al., unpubl. manuscript). If, on the other hand, the sequestration of PAs originated only once with a shift to asteraceous food plants in an ancestral *Oreina*, the secondary loss of this capacity remains to be explained. Experiments suggest that PA sequestration could be advantageous because it may be more effective than defense by cardenolides against

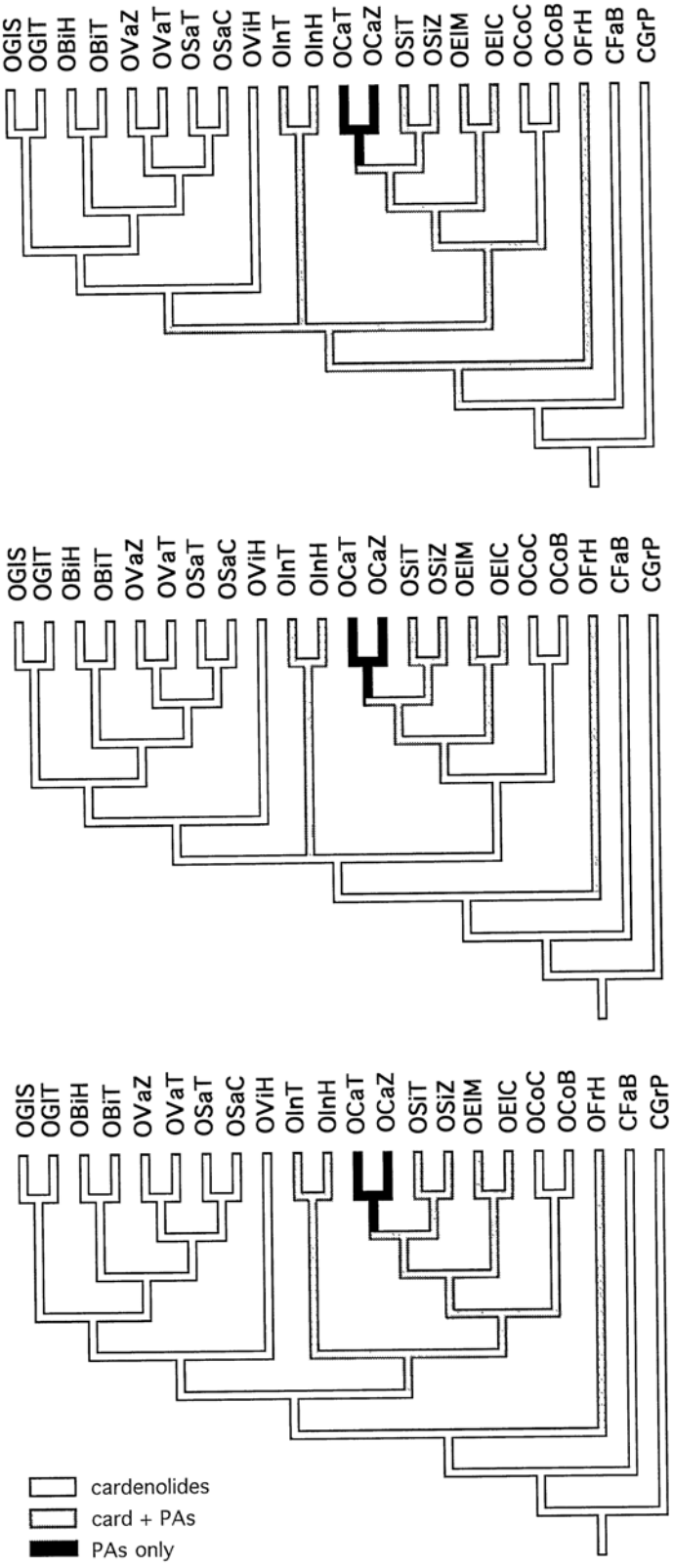

FIG. 6. Three equally parsimonious reconstructions for the evolution of chemical defense. *Oreina melanocephala* has been excluded from these trees as its defensive compounds have not yet been identified. (A) reversals favored over parallelisms, (B) parallelisms favored over reversals, (C) *O. intricata* positioned at the bottom of the cacaliae clade. For details, see text. Codes for beetle names and locations as in Table 1.

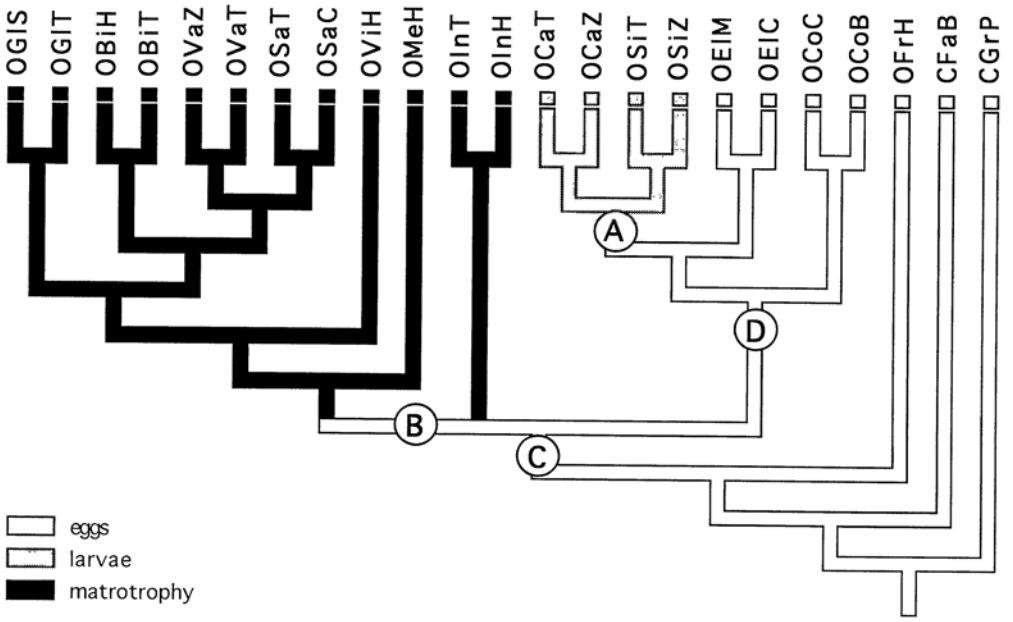

FIG. 7. Reconstruction of the evolution of the mode of reproduction. A, B, C, and D mark branches discussed in detail in the text. Codes for beetle names and locations as in Table 1.

avian predators (Rowell-Rahier et al. 1995) and *O. cacaliae*, which exclusively relies on PAs, is the most successful species in terms of local abundance and geographic range. However, a loss of the defensive plant compounds through host-plant switches might be compensated for by other factors. For example, in populations of *O. elongata* (Dobler and Rowell-Rahier 1994) and *O. speciosissima* (unpubl. obs.) and possibly in *O. frigida*, the exploitation of new resources and a broadening of the altitudinal distribution might have been more important than the maintenance of sequestration in the defensive strategy, especially because the beetles still dispose of autogenous cardenolides. This suggests that competition for food could be a critical factor in these species, many of which live in dense patches on their host plant during most of their life cycle (only pupation occurs in the soil; but both larvae and adults feed on the plants more or less continuously and move very little).

The fact that (1) the beetles were specialized and produced autogenous cardenolides before they shifted to toxic plants and (2) they are able to leave them again, shows that, at least in this case, protection through plant compounds was neither the reason for specialization (Bernays and Grahams 1988) nor is it necessarily an evolutionary dead end on the plants providing chemical protection.

Evolution of Reproductive Strategy

As discussed in the natural history section, the genus *Oreina* represents the full range of stages in the evolution of viviparity: oviparity, retention of the eggs in the oviducts until hatching with only yolk provided to the embryo, and retention of the eggs in the oviducts combined with matrotrophy by diffusion of nutrients through a reduced chorion (Bontems 1989). Here, we distinguish three states: oviparity, viviparity without matrotrophy, and viviparity with matrotrophy. To do so, we arbitrarily define the limit between the latter two states over the larval weight relative to the mother's weight: we will assume matrotrophy in species in which the newborn larvae weigh at least 1.1% as much as their mother. Apparently, the only structural modification associated with matrotrophy is partial (though never complete) reduction of the chorion (Bontems 1989). Thus, a definition via the relative weight of the larvae seems to be reasonable. The relative egg weights in the oviparous species range from 0.8% to 1% of the female weight, whereas this ratio reaches up to 3% in species with maternal nutrition (Table 2). Still, two viviparous species, *O. bifrons* and *O. virgulata*, have ratios just above our arbitrary limit indicating that we face a continuum between species with and without matrotrophy.

Mapping the three reproductive modes on our phylogeny (Fig. 7) leads to the conclusion that viviparity is a derived state while the production of eggs is the ancestral condition shared with the outgroup. However, viviparity must have evolved at least twice, once in the ancestor of *O. cacaliae* and *O. speciosissima* without additional nutrition of the developing larvae (clade [A]) and a second time in the ancestor of the clade including *O. melanocephala* and all descendants (B). Once more the evolutionary scenario remains simpler if the polytomy is resolved in a way that *O. intricata* is the sister taxon of *O. melanocephala*—in this case matrotrophy evolved only once. If, on the other hand, *O. intricata* is placed in the clade leading to *O. cacaliae*, then matrotrophy has either evolved twice independently (in *O. intricata* and in clade [B]) or once right after the branching of *O. frigida* (C) which would have been followed by a reversal to oviparity in the ancestor of *O. coerulea* and *O. elongata* (branch [D]) and a regain of viviparity this time without matrotrophy in the ancestor of *O. cacaliae* and *O. speciosissima* (A). Although all these reconstructions involve three steps, the last appears less likely than the two preceding, as a reversal from viviparity back to oviparity is assumed highly unlikely

(Blackburn 1992; Wourms and Lombardi 1992). A differing position of *O. frigida* as in the phenograms would increase the number of independent origins of viviparity or postulate exactly such a reversal.

Polyphyletic origins of viviparity are also known from reptiles (e.g., Blackburn 1992) and fishes (e.g., Wourms and Lombardi 1992; Meyer and Lydeard 1993). In beetles, viviparity is very rare, yet within one tribe of leaf beetles, the Chrysomelini, it occurs not only in *Oreina* but also in five other genera (15 times in *Chrysolina*, eight times in *Platyphora* Gistel, eight times in *Gonioctena* Chevrolat, once in *Paropsides* Motschulsky and twice in *Pyrgoides* Aslam; Bontems 1988). The apparent multiple origins of viviparity within this tribe of leaf beetles and its very rare occurrence in other beetle clades suggest a common preadaptation in the former, which facilitated its multiple independent adoption. In *Oreina*, no clear ecological advantage to this derived character (such as shortened extrautero developmental times or compensation for low fecundity) has so far been demonstrated (Dobler and Rowell-Rahier 1996). A comparison of larval survival and predation rates in species with different reproductive strategies is still under investigation (Rowell-Rahier et al., unpubl. manuscript).

Conclusions

Oreina species provide an example in which switches among host plants may rather be dictated by the available choice in high-altitude habitats than by plant relatedness and chemical similarity. In this genus, ecological factors seem to dominate historical commitments. Although it is suggestive that both autogenously produced defense and the state of free-living larvae freed the beetles of some constraints, we do not yet understand what role the different reproductive strategies play in host and habitat selection of these beetles. At least there is no obvious correlation with host switches or diet breadth.

The complex distribution and the various combinations of ecological and life-history characters in *Oreina* species preclude any simple scenario for the evolutionary history of each of these characteristics. For example, *O. frigida* combines a condition a priori assumed to be primitive (oviparity) with a seemingly derived one (sequestration) and a third (polymorphism in host family) that suggests the species' placement is between the two major groups of host association in the middle of the genus. Such complex combinations require parallelisms or reversals for some of the traits. Reconstructing the evolution of these traits on the cladogram actually results in a conservative estimate of the lability of these traits compared with differing positions of some species on the phenograms.

The evolutionary scenario we favor, because it simplifies the overall picture, involves an association with the Asteraceae at the base of the genus followed by two independent switches to Apiaceae and a loss of Asteraceae feeding in the gloriosa clade. The ancestral defensive strategy of the group clearly is the production of cardenolides, which is maintained throughout the genus with the exception of *O. cacaliae* and possibly *O. melanocephala*. We do not think that it is possible to decide yet whether the additional sequestration of pyrrolizidine alkaloids originated several times independently or only once followed by several losses later on. Additional physiological data on similarities or differences in PA metabolism and handling might provide further insights. For the evolution of reproductive strategy we assume that *O. intricata* is actually closer related to *O. melanocephala* and the gloriosa clade than to the cacaliae clade; in this case, two independent origins of viviparity are to be postulated, once in the ancestor of *O. cacaliae* and *O. speciosissima* without additional nutrition of the larvae, and once involving matrotrophy in the ancestor of *O. intricata* and all descendant species in this clade. Further investigation will attempt to clarify remaining questions of the biology and to include species from the genus *Chrysolina* to gain a broader picture of the amazing ecological flexibility these two genera express (Hsiao et al., unpubl. manuscript).

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