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Phylogenetics and evolution of the aphid genus *Uroleucon* based on mitochondrial and nuclear DNA sequences

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Abstract. The genus *Uroleucon*, and the related genus *Macrosiphoniella*, represent a large Tertiary radiation of aphids, with a total of about 300 species distributed throughout the world, primarily on host plant species in the family Asteraceae. A molecular phylogenetic study was conducted to identify major clades within *Uroleucon* and to address the cladistic validity of current subgeneric categories, the evolution of host plant associations, the age of origin, and intercontinental movements in this genus. The seventeen study species included members of the three major subgenera of *Uroleucon*, species from Europe and North America, one member of *Macrosiphoniella*, and two outgroups. Data consisted of DNA sequences for three mitochondrial regions and the nuclear gene EF1alpha, for a total of 4287 sites. Nodes supported strongly in both parsimony and maximum likelihood analyses suggest that: (1) Nearctic *Uromelan* are a monophyletic group branching near the base of the genus and not related to European *Uromelan*, (2) the New World subgenus *Lambersius* is possibly monophyletic but is not a tightly related group and is not closely related to other North American species, and (3) Nearctic members of subgenus *Uroleucon* are a closely related monophyletic group not allied with Nearctic *Uromelan* or *Lambersius*. Instead they represent a separate colonization by an Old World ancestor, as they are nested within a strongly supported clade containing European members of both subgenera *Uroleucon* and *Uromelan*. Neither of these subgenera is monophyletic. Molecular clock calculations, based on calibrations of mitochondrial divergences from other insects, suggest that *Uroleucon* + *Macrosiphoniella* is a relatively recent radiation, probably no more than 5–10 million years old. Although largely confined to Asteraceae, this clade did not radiate in parallel with its host plants. Rather, lateral movement between lineages of Asteraceae must have occurred repeatedly.

Introduction

Although aphids (Hemiptera: Aphidoidea) date to the Jurassic or earlier (Heie, 1987), much of modern species diversity in aphids is the result of radiation that occurred in the second half of the Tertiary (Heie, 1996). This relatively recent radiation is concentrated in the family Aphididae and is correlated with the diversification of herbaceous angiosperms, especially grasses (Poaceae) and composites (Asteraceae) in north temperate regions (Heie, 1996). The genus *Uroleucon* Mordvilko and close relatives, particularly the genus *Macrosiphoniella* del Guercio, are characteristic of this late phase in aphid evolution. *Uroleucon* and *Macrosiphoniella* are species-rich genera of host-specific aphids, most of which are confined to host plants in the large family Asteraceae or the related family Campanulaceae (e.g. Smith & Parron, 1978; Heie, 1995). There are 197 and 122 valid species of *Uroleucon* and *Macrosiphoniella*, respectively (Remaudiere & Remaudiere, 1997); almost certainly, others remain to be described. The Asteraceae, one of the largest plant families, and the Campanulaceae are among the numerous families of herbaceous angiosperms that became abundant in north temperate

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Table 1. Species included in the phylogenetic analysis. Outgroups were *Schizaphis graminum* (Aphidini, abbreviated Sg) and *Acyrthosiphon pisum* (Macrosiphini, abbreviated Ap). E = Europe, NA = North America. Ul. = *Uroleucon*, Um. = *Uromelan*, L. = *Lambersius*, M. = *Macrosiphoniella.*

Genus	Sub-genus	Species	Author	Native range	Host genus, higher taxon	Abbreviation
Ul.	*Um.*	*aeneum*	(Hille Ris Lambers)	E	*Carduus* (Asteraceae: Cardueae)	Uae
Ul.	*Ul.*	*ambrosiae*	(Thomas)	NA	*Ambrosia* (Asteraceae: Heliantheae)	Uam
Ul.	*Ul.*	*astronomus*	(Hille Ris Lambers)	NA	*Aster* (Asteraceae: Astereae)	Uas
Ul.	*L.*	*caligatum*	(Richards)	NA	*Solidago* (Asteraceae: Astereae)	Uc
Ul.	*L.*	*erigeronense*	(Thomas)	NA	*Erigeron* (Asteraceae: Astereae)	Ue
Ul.	*Um.*	*helianthicola*	(Olive)	NA	*Helianthus* (Asteraceae: Heliantheae)	Uh
Ul.	*Um.*	*jaceae*	(Linnaeus)	E	*Centaurea* (Asteraceae: Cardueae)	Uja
Ul.	*Ul.*	*jaceicola*	(Hille Ris Lambers)	E	*Centaurea* (Asteraceae: Cardueae)	Ujl
Ul.	*Ul.*	*obscurum*	(Koch)	E	*Hieracium* (Asteraceae: Lactuceae)	Uo
Ul.	*Um.*	*rapunculoidis*	(Borner)	E	*Campanula* (Campanulaceae)	Urp
Ul.	*Il.*	*rudbeckiae*	(Fitch)	NA	*Rudbeckia* (Asteraceae: Heliantheae)	Urd
Ul.	*Um.*	*rurale*	(Hottes & Frison)	NA	*Helenium* (Asteraceae: Heliantheae)	Urr
Ul.	*Um.*	*solidaginis*	(Fabricius)	E	*Solidago* (Asteraceae: Astereae)	Usl
Ul.	*Ul.*	*sonchi*	(Linnaeus)	E	*Sonchus* (Asteraceae: Lactuceae)	Usn
M.		*ludovicianae*	(Oestlund)	NA	*Artemesia* (Asteraceae: Anthemiidae)	Ml

regions as climates became cooler and drier beginning in the Oligocene (Muller, 1981).

The allegiance of most *Uroleucon* and *Macrosiphoniella* to Asteraceae typifies the host plant fidelity of many of the aphid groups arising during this late episode of aphid diversification on herbs. It contrasts with the promiscuity of certain other groups diversifying during the same period, notably the genus *Aphis* L., which contains approximately the same number of species as *Uroleucon* plus *Macrosiphoniella* but occurs on a comparatively vast number of plant families (Eastop, 1977). Individual species of *Uroleucon* are typically confined to one host plant species or to several plants within the same genus (Hille Ris Lambers, 1939; Moran, 1984). In some cases, species complexes are restricted to groups of closely related plants. For example, the *Uroleucon jaceae* complex occurs on genera within the tribe Cardueae, the *Uroleucon cichorii* complex occurs on genera in the tribe Lactuceae (Hille Ris Lambers, 1939), and North American species in the subgenus *Lambersius* Olive are concentrated on members of the tribe Astereae. These patterns of host plant affiliations within *Uroleucon* raise the possiblity of parallel diversification, i.e. that aphid speciation has occurred more or less synchronously with speciation of the corresponding host plants. A consistent pattern of cospeciation could be detected by comparing the reconstructed phylogenies of insects and hosts, as for the leaf beetles *Phyllobrotica*, for which evidence supports parallel diversification with hosts in the Lamiaceae (Farrell & Mitter, 1990), and *Ophraella*, for which phylogenetic evidence contradicts parallel diversification with lineages of Asteraceae (Funk *et al*., 1995).

Uroleucon has been subdivided into three major subgenera, primarily on the basis of three characters: pigmentation of the cauda, pigmentation of the siphunculi, and the colour in life (Hille Ris Lambers, 1939; Olive, 1965a; Heie, 1995). Species that are red, brown or black in life with uniformly dark siphunculi are classified in subgenus *Uroleucon* Mordvilko if the cauda is pale and in subgenus *Uromelan* Mordvilko if the cauda is pigmented. Species that are green in life with siphunculi pale basally are classified as *Lambersius* Olive (1965a). *Uroleucon* and *Uromelan* are distributed throughout the northern hemisphere, with some representatives in the southern hemisphere, especially South America (de Carvalho *et al*., 1998). *Lambersius* is confined to the New World and includes a number of South American representatives (Olive, 1965a; Robinson, 1986; de Carvalho *et al*., 1998). A number of red or brown species have intermediate caudal pigmentation and thus are not easily categorized under the current subgeneric framework (e.g. Olive, 1965b). Additionally, some other morphological characters appear to contradict the division between subgenera *Uroleucon* and *Uromelan*, suggesting that caudal pigmentation is homoplasious at the level of the genus. For example, most Nearctic species of all three subgenera are united in possessing pale coxae, in contrast to Old World species of both *Uroleucon* and *Uromelan*, most of which have brown or black coxae.

In this paper, a molecular phylogenetic dataset and analysis is presented for some representative species of *Uroleucon*. The aim of the study is to address the following interrelated questions about the evolution of this group. (1) Is *Uroleucon* monophyletic? In particular, do *Uroleucon* species have a common ancestor occurring after the split from *Macrosiphoniella*? (2) Which, if any, of the three major subgenera of *Uroleucon* are monophyletic? (3) What is the pattern of colonization between Old World and New World? (4) Can the nature of the shared history of *Uroleucon* and its host plants be characterized? For example, has *Uroleucon* diversified through cospeciation

Table 2. Collection data for species included in study. Full species names are in Table 1.

Species	Collection details
A. pisum	USA: cultured on *Vicia faba*, gift of D. Voegtlin
M. ludovicianae	USA: Arizona, Pima County, Mt Lemmon, 17 July 1996, *Artemisia ludoviciana*, N. Moran
S. graminum	biotype E, cultured on wheat, gift of P. Baumann
U. aeneum	Sweden: Rävsnäs, 8 April 1996, *Cirsium vulgare*, J. Sandström
U. ambrosiae	USA: Minnesota, St. Paul, 20 July 1995, *Ambrosia trifida*, N. Moran
U. astronomus	USA: Minnesota, Cross Lake, 20 July 1995, *Aster macrophyllus*, N. Moran
U. caligatum	USA: Minnesota, Cross Lake, 20 July 1995, *Solidago altissima*, N. Moran
U. erigeronense	USA: New Mexico, Albuquerque, 16 Nov 1995, *Conyza canadense*, N. Moran
U. helianthicola	USA: Minnesota, Cross Lake, 22 July 1995, *Helianthus* sp., N. Moran
U. jaceae	Sweden: Vätö, 20 July 1996, *Centaurea jacea*, J. Sandström
U. jaceicola	Sweden: Lund, 25 June 96, *Centaurea jacea*, J. Sandström
U. obscurum	Sweden: Vätö, 20 July 1996, *Hieracium* sp., J. Sandström
U. rapunculoidis	Sweden: Lund, 25 June 1996, *Campanula rapunculoides*, J. Sandström
U. rudbeckiae	USA: Arizona, White Mountains, 20 Aug 1996, *Rudbeckia hirta*, M. Kaplan
U. rurale	USA: Georgia, Athens, 20 June 1994, *Actinomeris alternifolia*, N. Moran
U. solidaginis	Sweden: Vätö, 20 July 1996, *Solidago virgaurea*, J. Sandström
U. sonchi	USA: Arizona, Tucson, July 1995, *Sonchus oleraceum*, N. Moran

with Asteraceae or has it radiated through colonization of existing host plant taxa? Has *Uroleucon* diversified over the same time period as its major host plants (the family Asteraceae)?

Materials and methods

Aphid samples

Due to the number of species in *Uroleucon* and the need for extensive sequence to achieve any phylogenetic resolution, only a very incomplete representation of species was possible. The selection of taxa was designed to address the above questions. Among included species were representatives of all three major subgenera, species native to both Europe and North America, and species from several tribes of Asteraceae and from Campanulaceae (Table 1). One species of *Macrosiphoniella* was included; this genus is presumed to be monophyletic based on morphological criteria (Hille Ris Lambers, 1938). *Acyrthosiphon pisum* (placed with *Uroleucon* and *Macrosiphoniella* in the tribe Macrosiphini) and *Schizaphis graminum* (in tribe Aphidini) were included as outgroups in order to establish the rooting of the *Uroleucon* + *Macrosiphoniella* clade. *Macrosiphoniella* was not designated as an outgroup, as prior to the analysis we could not exclude the possibility that it arose within *Uroleucon*.

Collection data are listed in Table 2. For about half of the species (*U. aenum, U. jaceae, U. jaceicola, U. obscurum, U. rapunculoides, U. sonchi, M. ludovicianae, A. pisum, S. graminum*), aphids were grown in the laboratory on potted host plants. In these cases, colonies were initiated with a single female and are expected to contain DNA from genetically homogeneous individuals. The other samples were collected in the field from a single colony or from neighbouring colonies. Voucher specimens are deposited with the United States National Museum, Beltsville, Maryland, U.S.A. Aphids were frozen at –80°C until extraction. For each species, genomic DNA was extracted from 0.1–2.0 g of aphids, using routine methods as described in Rouhbakhsh *et al.* (1996).

Sequence selection and determination

DNA sequences were used from three mitochondrial regions and one nuclear gene; fragments were generated for sequencing using the polymerase chain reaction (PCR; Saiki *et al.*, 1988). Genes included in each region and the primers used for PCR amplification are listed in Table 3. Primers for mitochondrial fragments were from the Insect Mitochondrial DNA Primer Oligonucleotide Set obtained from the University of British Columbia Nucleic Acid-Protein Service Unit; these primers are also discussed in Simon *et al.* (1994). Primers for the EF1alpha fragment were designed and provided by Dr Ben Normark. All PCR reactions were performed in 50 μl volumes with 1X PCR buffer (Gibco/BRL, Rockville, Maryland, U.S.A.), 0.25 mM of each dNTP, 0.4 pmol/ml of each primer, 0.4 ng/ml of genomic DNA, and 2 units of Taq DNA polymerase (Gibco/BRL). $MgCl_2$concentrations and primer annealing temperatures were optimized for the different fragments: 2.5 mM $MgCl_2$ and 52°C for 12S/16S, 1.5–3.5 mM $MgCl_2$ and 60–64°C for COI/COII (depending on the taxon), 2.5–4.0 mM $MgCl_2$ and 56°C for NADH1. For amplification of EF1alpha, an alternate PCR buffer was used (Stratagene, La Jolla, California, U.S.A., Buffer #6: 10 mM Tris-HCl pH 8.8, 1.5 mM $MgCl_2$, 75 mM KCl). For some taxa, it was necessary to supplement $MgCl_2$ to 3.5 mM and to vary annealing temperature between 50 and 52°C. The cycling parameters for all genes were 30 cycles of 94°C (1 min), annealing temperature (1 min), 72°C (1 or 2 min). Yields were improved for EF1alpha

Table 3. Primers for amplification of DNA sequences used for *Uroleucon* phylogeny. Names correspond to those used in Simon *et al*. (1994). Names in parentheses correspond to University of British Columbia listings.

Genes (partial)	Forward primer	Reverse primer	Fragment size (portion sequenced)
12S/16S rRNA	16sa (mtD-33) 5′-ATGTTTTTGTT -AAACAGGCG-3′	12sai (mtD-36) 5′-AAACTAGGATTAGAT -ACCCTATTAT	1140 bp (all)
NADH1	ND1 (mtD-30) 5′-GTAGCATTTTTAACTTT -ATTAGAACG-3′	CB1 (mtD-26) 5′-TATGTACTACCATGAGG -ACAAATATC-3′	1660 bp (558 bp)
COI/COII	Jerry (mtD-08) 5′-CAACATTTATTTT -GATTTTTTGG-3′	Barbara (mtD-18) 5′-CCACAAATTTCTGAA -CATTGACCA-3′	1462 bp (all)
EF1alpha	EFS175 5′-GGAAATGGGAAAA -GGCTCCTTCAAGTAYGCYTGGG-3′	EF2 5′-ATGTGAGCAGTGTGG -CAATCCAA-3′	1118 bp (all)

and NADH1 by adding 16 ng/ml of the T4 gene 32 protein (Ambion, Austin, Texas, U.S.A.). For each fragment, 150 ml of PCR product was purified for sequencing using the QIAquick spin PCR kit (Qiagen, Valencia, California, U.S.A.). Purified PCR products were resuspended in water and sent to the Laboratory of Molecular Systematics and Evolution Automated Sequencing Facility (University of Arizona). Automated DNA sequencing was performed for each fragment using the same primers from both ends of the amplified fragments plus internal primers in the case of EF1alpha. Sequences were checked using Sequence Navigator (Applied Biosystems, Inc., Foster City, California, U.S.A.). Ambiguous bases were coded as unknown and treated as missing data in phylogenetic analyses. The following sequences were not obtained due to technical difficulties: COI/COII for *U. solidaginis*, NADH1 for *U. jaceicola* and part of EF1alpha for *A. pisum*; these were coded as missing in analyses. Sequences were deposited in GenBank under accession numbers AF068464-AF068480 (EF1alpha), AF069097-AF069113 (12S/16S), AF057045-AF057060 (NADH1) and AF059684-AF059699 (COI/COII).

Alignment

Coding regions were identified and translated and amino acid sequences aligned. DNA alignments were then fitted to the amino acid alignments, which were unambiguous. Noncoding regions, including rRNA, spacers, tRNA, and introns, were aligned using PILEUP in the GCG package (Genetics Computer Group, 1997) and then adjusted by hand. Because overall divergences were low, these alignments were largely unambiguous.

Analysis

Analyses were conducted using test version 4.0.0d61 of PAUP*, written by David L. Swofford. Maximum parsimony analyses were run, with sites weighted equally. Bootstraps analyses were used to determine strength of support for individual nodes, with 1000 heuristic search replicates for each analysis. Mitochondrial and nuclear sequences were analysed separately and together. In addition, analyses were run using the Likelihood option of PAUP*. In the maximum likelihood analyses, base frequencies were set to empirical levels, substitution rates were variable among sites according to a gamma distribution with shape parameter of 0.5 and 4 rate categories, and the Hasegawa–Kishino–Yano model of substitution was selected. A molecular clock was not enforced.

Results and discussion

Phylogenetic trees resulting from parsimony analyses of mitochondrial genes, the nuclear gene, and the combined dataset containing mitochondrial and nuclear sequences are shown in Fig. 1. For each of the three datasets, a single most parsimonious tree was found. In each case, this tree was in broad agreement with the maximum likelihood tree. Additionally, there was no major conflict between trees obtained from the three datasets: nodes with strong bootstrap support from one analysis are never contradicted with strong support for a conflicting node in one of the other two analyses. However, the two datasets differ in level of resolution provided for particular nodes, as discussed below. Despite lack of resolution for some nodes and the meager representation of *Uroleucon* species, several findings emerge from the phylogenetic analysis, allowing most of our initial questions to be addressed.

Is Uroleucon *monophyletic?*

As expected given this set of species, there is strong support for placement of *Macrosiphoniella* with *Uroleucon* (Fig. 1). Additionally, the *Macrosiphoniella* representative branches outside of *Uroleucon*, supporting monophyly of the latter. Although *Macrosiphoniella* is a possible sister group to the genus *Uroleucon*, other taxa, such as the genus *Obtusicauda*,

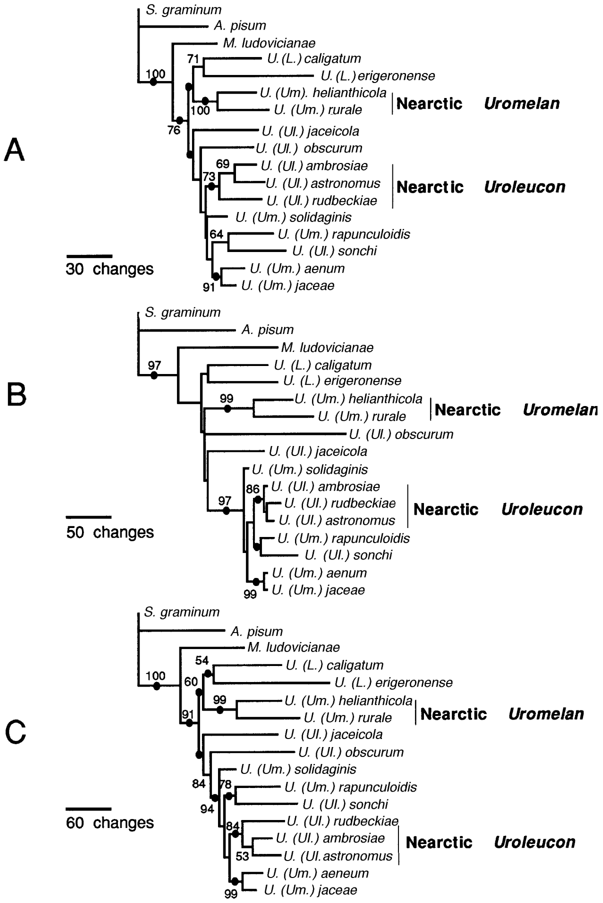


Fig. 1. Phylogenies based on maximum parsimony analyses of DNA sequences. Bootstrap support based on 1000 replicates is indicated for nodes with greater than 50% support. Nodes also present in maximum likelihood trees are marked with a disc. A, phylogeny based on combined mitochondrial sequences, totalling 3169 nucleotides with 348 parsimony informative sites and length equal to 1330; B, phylogeny based on partial sequence of the nuclear gene EF1alpha including both exons and introns, totalling 1118 nucleotides, with eighty-nine parsimony informative sites and length equal to 412; C, phylogeny based on both mitochondrial and nuclear sequences, totalling 4287 nucleotides with 437 parsimony informative sites and length equal to 1754. L. = subgenus *Lambersius*; Ul. = subgenus *Uroleucon*; Um. = subgenus *Uromelan*. The scale bars for branch lengths correspond to the minimum numbers of reconstructed changes.

also show a close relationship. Thus, the sister clade to the genus *Uroleucon* cannot be determined from our analysis.

Are the major subgenera of Uroleucon *monophyletic?*

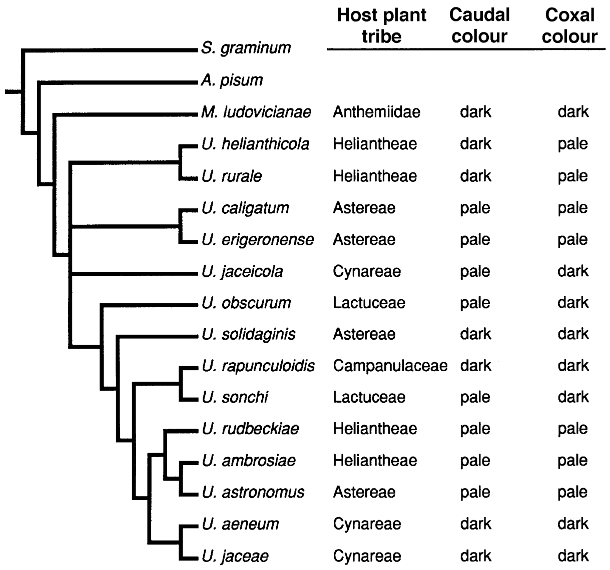
Subgenera Uroleucon *and* Uromelan. Neither subgenus *Uroleucon* nor subgenus *Uromelan* is monophyletic, a conclusion strongly supported by separate and combined analyses of mitochondrial and nuclear regions. In the result from the combined data, a clade of eight species consists of species placed in both subgenus *Uroleucon* and subgenus *Uromelan* (Fig. 1C: clade including *U. astronomus, U. ambrosiae, U. rudbeckiae, U. aenum, U. jaceae, U. solidaginis, U. sonchi, U. rapunculoidis*). This clade is strongly supported by the nuclear data and the combined analysis and weakly supported by the mitochondrial data (Fig. 1). Additionally, species from both subgenera *Uroleucon* and *Uromelan* fall outside this clade. Thus, neither subgenus is monophyletic, and caudal colour, the basis for the *Uroleucon*/*Uromelan* division, is a homoplasious character at the level of the genus (Fig. 2).

Nearctic Uroleucon. The three Nearctic representatives of the subgenus *Uroleucon* form a strongly supported monophyletic group that is nested within the clade of eight species mentioned above. Otherwise, this clade contains European species. This placement and monophyly of the Nearctic *Uroleucon* is strongly supported by both mitochondrial and nuclear sequences (Fig. 1). The three species were chosen as diverse representatives of the North American members of the subgenus. For example, *pseudochrysanthemi* (Oestlund), *ciefi* (Olive), *gigantiphagum* Moran, *lanceolatum* (Patch), *nigrotibium* (Olive), *nigrotuberculatum* (Olive), *olivei* Moran, *paucosensoriatum* (Hille Ris Lambers), *pieloui* (Richards) and *sijpkensi* Hille Ris Lambers all appear to be as similar or more similar to *astronomus* than do either *ambrosiae* or *rudbeckiae* (Moran, 1984; Robinson, 1985). In particular, morphological traits of *rudbeckiae* are quite divergent from those of other New World *Uroleucon*. Thus, the strongly supported clade, *ambrosiae* + *astronomus* + *rudbeckiae*, represents a large monophyletic group that includes most or all red or brown species with pale caudae that are endemic to North America.

New World Uromelan. A close relationship of the two included species of New World *Uromelan* is strongly supported by both mitochondrial and nuclear genes (Fig. 1). These are not closely related to Old World *Uromelan*, such as *U. solidaginis* or *U. jaceae*, nor are they close to other New World *Uroleucon*. Several other observations suggest monophyly of a set of North American *Uromelan*, including, in addition to *U. helianthicola* and *U. rurale, U. eupatorifoliae, U. illini, U. parvotuberculatum, U. tardae, U. tuatuaie* and *U. verbesinae*. Most of these species are confined to members of the tribe Heliantheae, and all possess a combination of morphological features not seen in any other members of the genus: pale coxae, anal plate and genital plate but darkly pigmented cauda (Fig. 2). Whereas all Old World *Uromelan* possess sclerotized spots at the bases of dorsal abdominal hairs (Hille Ris Lambers, 1939), abdominal sclerites are frequently pale or absent in these Nearctic *Uromelan* (e.g. Olive, 1963; Robinson, 1985).

Uroleucon jaceae *group*. A close relationship of the European species, *U. jaceae* and *U. aeneum*, is strongly indicated by both mitochondrial and nuclear genes. These species are members of a complex restricted to plants in the tribe Cardueae (Hille Ris Lambers, 1939).

The subgenus Lambersius. Of the three major subgenera of *Uroleucon*, only *Lambersius* is possibly monophyletic, but



	Host plant tribe	Caudal colour	Coxal colour
S. graminum			
A. pisum			
M. ludovicianae	Anthemiidae	dark	dark
U. helianthicola	Heliantheae	dark	pale
U. rurale	Heliantheae	dark	pale
U. caligatum	Astereae	pale	pale
U. erigeronense	Astereae	pale	pale
U. jaceicola	Cynareae	pale	dark
U. obscurum	Lactuceae	pale	dark
U. solidaginis	Astereae	dark	dark
U. rapunculoidis	Campanulaceae	dark	dark
U. sonchi	Lactuceae	pale	dark
U. rudbeckiae	Heliantheae	pale	pale
U. ambrosiae	Heliantheae	pale	pale
U. astronomus	Astereae	pale	pale
U. aeneum	Cynareae	dark	dark
U. jaceae	Cynareae	dark	dark

Fig. 2. Host plant affiliations and pigmentation characteristics of *Uroleucon* in relation to inferred phylogenetic relationships. Host plant tribe, caudal pigmentation and coxal pigmentation are all homoplasious.

monophyly of *Lambersius* is not strongly supported (Fig. 1). The two included species, *erigeronense* and *caligatum*, do not appear to be closely related: genetic distances between them are approximately as great as distances from each to other species of the genus *Uroleucon* and approximately the same as for distances between *Macrosiphoniella* and *Uroleucon* species (Table 4). These observations suggest that divergence of these two *Lambersius* species occurred early in the history of the genus. Certain pale green South American species, such as *Uroleucon chilense* (Essig) and *Uroleucon bereticum* (Blanchard), not included in this study are possibly even more divergent based on morphometric analyses (de Carvalho *et al.*, 1998). These may reflect older splits within *Lambersius*, or they may represent an entirely different clade of green *Uroleucon*. However, monophyly of *Lambersius* is supported by shared cuticular pigmentation characteristics, in addition to the green colour in life; most notably, all have pale bases of the siphunculi, a feature otherwise rare in *Uroleucon* (Moran, 1984; Robinson, 1986).

What is the pattern of colonization between Old World and New World?

Parsimony results from EF1alpha and from the combined dataset do not contradict a sister-group relationship of New World *Uromelan* with *Lambersius* (Fig. 1B,C). Such a relationship, which is only weakly supported by our results, is consistent with the possibility that a common ancestor of New World *Uromelan* and *Lambersius* colonized America and diversified there, giving rise to two subclades: a mostly darkly pigmented radiation concentrated on hosts of the tribe Heliantheae (*Uromelan*) and a green group concentrated on the tribe Astereae (*Lambersius*). Aside from recent introductions of *Lambersius* species to Europe and Asia, both groups are restricted to the New World. *Lambersius* is distributed throughout North and South America (Robinson, 1985, 1986; de Carvalho *et al.*, 1998).

Distances between the two *Lambersius* included in this study, and between *Lambersius* and the two Nearctic *Uromelan*, are about as large as those between *Lambersius* and other *Uroleucon* species (Table 4). These results suggest that the American species of *Lambersius* and *Uromelan* have been diversifying for much of the history of the genus and do not represent recent colonizations followed by radiation. In contrast, Nearctic *Uroleucon*, represented in this study by *ambrosiae* + *astronomus* + *rudbeckiae*, appear to be the result of a more recent colonization and subsequent radiation, based on the low genetic distances among them (Table 4) and on their inclusion in a clade otherwise consisting of closely related European species (Fig. 1).

Reconstruction of the historical biogeography of the genus is not possible due to the limited taxon sampling. However, any interpretation supports at least two migrations between the Old and the New World.

Has Uroleucon *diversified over the same period as Asteraceae?*

The deepest divergences within *Uroleucon* and between *Uroleucon* and *Macrosiphoniella* show genetic distances from about five to ten substitutions per 100 sites for the four mitochondrial genes (Table 4). For several other arthropod groups, the rate of substitution for a variety of mitochondrial genes has been calibrated at about 2% per million years (Brower, 1994; Funk *et al.*, 1995; Juan *et al.*, 1996). Under the assumption that this rate applies to *Uroleucon*, the divergences of Table 4 would place the age of *Uroleucon* at less than 5 million years. This timing agrees with the hypothesis of Heie (1996) that the radiations of Aphididae on Asteraceae (and Poaceae) appeared during or after the Miocene. Thus, *Uroleucon* appears to have radiated after pollen of several tribes of Asteraceae became abundant in the fossil record, ≈ 20 million years ago (Muller, 1981), and considerably after the origin of the Asteraceae, which occurred during the Eocene or earlier (DeVore & Stuessy, 1995; Bremer & Gustafsson, 1997).

The conclusion that *Uroleucon* diversified during the past 5 million years depends on the premise that rates are not slower in *Uroleucon* than in the insects which were used to obtain the calibration. If so, the distances in *Uroleucon* would represent older splits than the same distances in other insects. However, calculations of pairwise divergences among aphid and other insect mitochondrial sequences indicate that rates in aphids generally are similar or somewhat faster than rates in other insects. Another possible basis of underestimation of divergence dates is

Table 4. Pairwise genetic distances between selected taxa or clades. Values are the mean of the pairwise distances for comparisons between clades containing more than one taxon; minimum and maximum distances for these comparisons are given in parentheses. Distances are numbers of substitutions per 100 sites, corrected for multiple substitutions at a site with the Kimura two-parameter model. Abbreviations are given in Table 1.

Pairwise comparison	12S-16S	CO1	CO2	ND1	EF1alpha
Between other Macrosiphini and *Uroleucon*: Ap – (Ml + *Uroleucon*)	6.1 (4.6–10.0)	7.36 (6.2–9.6)	9.54 (8.2–10.9)	8.1 (6.2–11.6)	4.98 (4.3–7.3)
Between *Macrosiphoniella* and *Uroleucon*: Ml – *Uroleucon*	4.2 (2.8–8.4)	6.0 (5.0–8.0)	5.7 (4.3–6.9)	7.2 (5.8–12.4)	4.2 (3.5–5.4)
Between *Lambersius* and other *Uroleucon*: (Ue + Uc) – other *Uroleucon*	5.6 (3.6–11.2)	5.5 (3.6–8.0)	6.4 (4.3–7.6)	8.5 (4.8–12.6)	3.0 (1.9–4.7)
Within *Lambersius*: (Ue – Uc)	7.3	4.8	5.1	10.6	3.4
Between NW *Uromelan* and other *Uroleucon*: (Uh + Urr) – other *Uroleucon*	4.5 (2.5–8.9)	6.8 (5.5–9.8)	6.8 (5.6–9.1)	7.1 (4.8–10.6)	3.8 (3.0–6.2)
Within North American *Uromelan*: Uh – Urr	1.6	5.9	3.3	4.4	3.4
Within members of clade (Uam & Uas & Urd) – (Uae & Uja) – Urp – Usn – Usl	2.2 (0.8–3.3)	4.1 (2.7–7.2)	4.5 (2.9–6.0)	4.9 (2.6–8.0)	1.1 (0.7–1.6)
Within *Uroleucon jaceae* complex: Uae – Uja	1.1	0.9	2.4	2.6	0.0
Within NW clade of subgenus *Uroleucon*: Uam – Uas – Urd	1.6 (1.4–1.9)	3.6 (2.1–4.7)	3.6 (2.2–4.5)	3.6 (2.9–4.4)	0.7 (0.5–0.9)

underestimation of distances due to saturation at some sites. However, for the three coding mitochondrial genes within *Uroleucon*, the mean divergences for the *Macrosiphoniella*-*Uroleucon* split are 25–27% for synonymous sites and 1–5% for non-synonymous sites, suggesting that saturation is not a major problem. Interpreted conservatively, the mitochondrial distances provide compelling support of Heie's (1996) view that diversification of *Uroleucon* occurred during the past 20 million years, after the major groups of Asteraceae were established. Nonetheless, the possibility that South American *Uroleucon* species represent basal lineages, suggested by the distinctive morphology of some species (Essig, 1953; Delfino, 1994; de Carvalho *et al*., 1998), is intriguing in view of evidence that the origin and early diversification of the Asteraceae took place in South America (Bremer, 1993; Bremer & Gustafsson, 1997) and of the fact that *Uroleucon* is peculiar among Aphididae in having a diverse representation of endemic species anywhere in the southern hemisphere (Heie, 1994; de Carvalho *et al*., 1998).

Has Uroleucon *diversified through cospeciation with Asteraceae or has it radiated through horizontal movement among host taxa?*

These results indicate clearly that *Uroleucon* lineages have shifted repeatedly among different tribes of Asteraceae. Often, a single host tribe is used by distantly related *Uroleucon* species. Conversely, closely related *Uroleucon* may use different tribes. Inclusion of additional species would vastly increase the number of required colonization events, for example, *Uroleucon pepperi* (Olive, 1965b) is a Nearctic *Uroleucon* that, on morphological grounds, is clearly a member of the *ambrosiae + astronomus + rudbeckiae* complex, although it feeds on *Cirsium* within the Cardueae, the tribe used by *U. aeneum*, *U. jaceae*, and the more distantly related *U. jaceicola*. Members of this Nearctic *Uroleucon* complex, which must be very young because it is nested within a larger clade that shows very low internal divergences (Table 4), feed on a wide diversity of Asteraceae including: Astereae, e.g. *astronomus, lanceolatum, nigrotibium, nigrotuberculatum* and *olivei* Moran; Heliantheae, e.g. *ambrosiae, rudbeckiae* and *obscuricaudatum* (Olive); Cardueae, e.g. *pepperi*; Eupatoriae, e.g. *ciefi*; Lactuceae, e.g. *hieracicola* (Hille Ris Lambers) and *pseudambrosiae* (Olive); Inulae, e.g. *russellae* (Hille Ris Lambers). Fossil pollen records, combined with phylogenetic knowledge, indicate that these tribes diverged by the mid-Oligocene (Muller, 1981), implying that this diversity of host associations must have originated through acquisition of existing host taxa.

Campanulaceae is related to Asteraceae and one interpretation of the fact that *Uroleucon* uses both families is that a *Uroleucon* ancestor colonized a common ancestor of Campanulaceae and Asteraceae with descendant aphid lineages retaining one or the other host family. This interpretation is contradicted by several observations from the current study that imply that use of Campanulaceae represents a derived condition within the genus *Uroleucon*. First, these two plant families diverged in the Eocene or earlier (Bremer & Gustafsson, 1997), long before the diversification of *Uroleucon* based on molecular clock estimates. Second, the ancestral host for *Macrosiphoniella* and *Uroleucon* was probably Asteraceae, as the large majority of species in both aphid genera (and also *Obtusicauda*) feed primarily on this family. Finally, *U. rapunculoidis*, which feeds on *Campanula*, is nested within a well supported clade represented by eight species, which otherwise feed on Asteraceae (Fig. 1). In fact, these phylogenetic results suggest a relationship between European species feeding on Lactuceae and species feeding on Campanulaceae, a possibility that has been suggested on the basis of morphological criteria (J. Holman, personal communication).

General discussion

Non-monophyly of established subgenera

Of the three major recognized subgenera of *Uroleucon*, only *Lambersius* is possibly monophyletic; if monophyletic, *Lambersius* is not a tightly related group. However, Nearctic members of subgenus *Uromelan* and Nearctic members of subgenus *Uroleucon* are each likely to be monophyletic groups. Nearctic *Uromelan* are rather distant from other members of the genus, but show a possible relationship to *Lambersius*. Nearctic species in subgenus *Uroleucon* form a clade nested within a clade containing European species in subgenus *Uroleucon* + *Uromelan* (Fig. 1). Caudal and coxal pigmentation are both homoplasious characters for this set of taxa, although each changes only a few times on the tree; the same is true of pigmentation of the bases of the siphunculi (pale in both *Lambersius* and *rudbeckiae*; Fig. 2)

Levels of pigmentation of coxae, anal plate and genital plate are strongly correlated yet independent of levels of pigmentation of other parts, such as cauda, abdominal scleroites, antennae and tibiae. Most Old World species of either *Uroleucon* or *Uromelan* have darkly pigmented coxae, anal plate and genital plate; in contrast, most American *Lambersius*, *Uroleucon* and *Uromelan* share the feature of pale coxae, anal plate and genital plate. This similarity between Nearctic representatives of the three subgenera appears to result from convergence. Several species from western North America and not included in our study, including *U. deltense* Robinson, *U. eoessigi* (Knowlton) and *U. vancouverense* Robinson, have dark coxae (Robinson, 1985); their placement in relationship to other American species is not clear.

Speciation mode in Uroleucon

Can these results elucidate how this clade, among the most species-rich radiations of aphids, has diversified? Apparently, the radiation of *Uroleucon* has been accompanied by relatively few movements between continents and many movements among existing lineages of Asteraceae and Campanulaceae (Fig. 2). Most aphid populations are extremely mobile and probably require a large geographical barrier to effect reproductive isolation between species. At the same time, our molecular clock estimates of age indicate that *Uroleucon* diversity arose quickly, probably in the past 10 million years. Transoceanic colonization events are apparently few and unnecessary for cladogenesis, suggesting that subdivisions of species' ranges are too infrequent for *Uroleucon* species to have arisen primarily through allopatric speciation. For example, most of the forty-four North American species assigned to subgenus *Uroleucon* (represented by *U. ambrosiae*, *U. astronomus* and *U. rudbeckiae* in this study) have ranges corresponding to large portions of the area of North America and apparently coincident with host ranges (Robinson, 1985). Yet this group of species must have radiated rapidly following colonization of North America: mitochondrial divergences among these three North American species and between these and related European species suggest that the colonization of North America occurred only about 2 million years ago (Table 4). As noted, host associations of North American members of subgenus *Uroleucon* clearly have evolved through repeated colonization of existing lineages of Asteraceae. Finally, *Uroleucon* species are highly host specific and mate on hosts. The most plausible view of their radiation is that the acquisition of a new host occasionally leads to reproductive isolation in sympatry and the consequent formation of new species.

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