

THE ORIGIN OF WORKERLESS PARASITES IN
LEPTOTHORAX (S.STR.) (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

The evolutionary origin of workerless parasitic ants parasitizing colonies of *Leptothorax* (s.str.) is investigated using data on morphology, chromosome number, and allozyme phenotype of both social parasites and their hosts. Of the three previously proposed pathways, the evolution of workerless parasites from guest ants or slave-makers is unlikely, at least according to a phenogram obtained by UPGMA clustering of Nei's similarities based on seven enzymes. Intraspecific evolution of the workerless parasites *Doronomyrmex goesswaldi*, *D. kutteri*, and *D. pacis* from their common host, *Leptothorax acervorum* cannot be excluded with the present data. The workerless parasite *L. paraxenus*, however, clearly differs from its host, *L. cf. canadensis*, in morphology and biochemistry, and most probably did not evolve from the latter species. It is proposed to synonymize *Doronomyrmex* under *Lepthorax* (s.str.).

INTRODUCTION

Eusocial insects by definition are characterized by a division of labor between non-reproductive workers and reproductive queens. Nevertheless, in a small minority of ant, bee, and wasp species, the worker caste has been secondarily lost. Instead of founding their own colonies solitarily, the queens of these workerless social parasites invade the nests of other, often closely related host species and exploit the present worker force to rear their own young. In

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Manuscript received 19 January 1996.

ants, the queens of some parasite species kill all host queens ("murder parasites," Faber, 1969), but in other species parasite and host queen live and reproduce together (inquilines in the strict sense, e.g., Bourke and Franks, 1991). The evolution of social parasites and in particular of socially parasitic ants has been extensively discussed starting with Darwin (1859). Three main routes leading to workerless parasitism have been proposed: workerless parasites might evolve a) directly from the species or species group serving them as host (Emery, 1909; Wasmann, 1909; Kutter, 1969; Buschinger, 1990; Bourke and Franks, 1991); b) from other parasites, such as temporary parasites, slave-makers, or guest-ants (Wasmann, 1908, 1909; Emery, 1909; Wilson, 1971); or c) from non-parasitic ancestors other than the host species (West-Eberhard, 1990; Bourke and Franks, 1991).

The myrmicine tribe Formicoxenini (formerly Leptothoracini, Bolton, 1994) is extraordinarily rich in social parasites and thus provides an ideal system to investigate the evolutionary pathways to workerless parasitism (Buschinger, 1986, 1989, 1990). *Leptothorax* (s.str.) (i.e., *L. acervorum*, *L. muscorum*, *L. cf. canadensis* and several other non-parasitic taxa), *Formicoxenus*, *Harpagoxenus*, and the palaearctic *Doronomyrmex* appear to be especially closely related (Buschinger, 1981, 1987) and have been grouped in a distinct subtribe within the Formicoxenini (Loiselle, Francoeur and Buschinger, 1990). They nevertheless exhibit remarkably different life histories. *Formicoxenus* are guest-ants, which live in the nests of *Formica*, *Myrmica*, or *Manica* (Francoeur et al., 1985). *Formicoxenus* workers beg food from their hosts but rear their own brood in separate chambers close to the host. The host colonies remain intact and continue to produce sexual brood (e.g., Wheeler, 1910). *Harpagoxenus* are slave-makers, whose queens after invading a *Leptothorax* (s.str.) host colony kill or expel all adult residents. *Leptothorax* workers which eclose from the conquered brood serve as "slaves" and take care of the slave-maker queen's larvae. *Harpagoxenus* workers eventually pillage brood from neighboring *Leptothorax* nests, which after eclosion serve as additional slaves. *Doronomyrmex kutteri* and *D. pacis* are workerless parasites which tolerate the *Leptothorax* host queens, though they probably decrease host reproductive success by feeding on their eggs (Kutter, 1969; Franks et al., 1990). *D. goesswaldi*, *L. paraxenus*, and *L. wilsoni* are workerless parasites which kill the host

queen, but not the adult host workers (Buschinger and Klump, 1988; Heinze, 1989; Heinze and Alloway, 1991).

Several attempts have been made to deduce the evolutionary origin of formicoxenine murder parasites and inquilines from data on morphology, karyotype, and enzyme phenotype (Buschinger, 1981, 1990; Heinze, 1991). Reviewing these previously published results and providing additional unpublished data, here I critically examine the hypotheses on the evolution of workerless parasites in this group and provide evidence that routes a and c are the most likely pathways leading to workerlessness.

MATERIALS AND METHODS

Colonies of parasitic and non-parasitic Formicoxenini (Table 1) were collected during the last 10 years in various parts of North America, Europe, and Turkey. Ants used in this study are from the following sites. Parasites: *L. paraxenus*: Bic (Co. de Rimouski, Québec), Milton (Halton Co., Ontario); *L. wilsoni*: Mt. Monadnock (Cheshire Co., New Hampshire), Escoumins (Co. de Saguenay, Québec), Jasper Nat. Park (Alberta; Buschinger and Schumann, 1994); *Doronomyrmex goesswaldi*: La Villette (Dept. Hautes-Alpes, France); *D. kutteri*: Leinburg (Bavaria, Germany); *D. pacis*: La Villette (Dept. Hautes-Alpes, France); *Harpagoxenus sublaevis*: Rudolstadt (Thuringia, Germany); *Formicoxenus quebecensis*: Waswanipi (Co. de Abitibi, Québec), Mt. du Lac des Cygnes (Co. de Charlevoix-Est, Québec), Jasper Nat. Park (Alberta; Buschinger, Schumann and Heinze, 1994). Non-parasitic species: *L. sp. A*: Tadoussac (Co. de Saguenay, Québec); *L. acervorum*: Grossostheim (Bavaria, Germany), Leinburg (Bavaria, Germany), Ilgaz Dagı Geçidi (Çankiri, Turkey); *L. cf. canadensis*: Bic (Co. de Rimouski, Québec), Tadoussac (Co. de Saguenay, Québec), Mount Monadnock (Cheshire Co., New Hampshire); *L. gredleri*: Sommerhausen (Bavaria, Germany); *L. muscorum*: Leinburg (Bavaria, Germany), Ilgaz Dagı Geçidi (Çankiri, Turkey); *L. "muscorum"* C: Maligne Canyon (Alberta); *L. retractus*: St. Siméon (Co. de Charlevoix-Est); *L. sphagnicolus*: L'Ascension (Co. de Chicoutimi, Québec); *D. pocahontas*: Maligne Canyon (Alberta). Details on the collecting procedure, laboratory rearing, and the life histories and collecting sites are published elsewhere (Heinze, 1989, 1993; Heinze and Ortius, 1991; Heinze, Trunzer, Lechner and Ortius, 1995).

Table 1. Synopsis of guest-ants, slave-makers, murder parasites, and queen-tolerantinquilines thought to be related to the ant subgenus *Leptothorax* (s.str.).

Species	Type	Host species	Geographical range	References
<i>Doronomyrmex goesswaldi</i>	murder parasite	<i>L. acervorum</i>	Alps	Buschinger and Klump, 1988
<i>D. kutteri</i>	inquiline	<i>L. acervorum</i>	Alps, S. Sweden, Estonia	Buschinger, 1971
<i>D. pacis</i>	inquiline	<i>L. acervorum</i>	Alps	Buschinger, 1971
<i>L. faberi</i>	murder parasite?	<i>L. cf. canadensis</i>	Maligne Lake, Alta.	Buschinger, 1982
<i>L. paraxenus</i>	murder parasite	<i>L. cf. canadensis</i>	Ontario, Québec	Heinze and Alloway, 1991
<i>L. wilsoni</i>	murder parasite	<i>L. cf. canadensis</i> , <i>L. sp. A</i>	Québec, New Brunswick, New Hampshire, Rocky Mountains	Heinze, 1989, Heinze et al. 1995, Buschinger and Shumann, 1994
<i>Harpagoxenus sublaevis</i> and <i>H. canadensis</i>	slave-maker	<i>L. acervorum</i> , <i>L. canadensis</i> , <i>L. gredleri</i> , <i>L. muscorum</i> , <i>L. sp. A</i>	coniferous forests in Northeastern North America and Eurasia	Buschinger, 1971; Heinze, Stuart, Alloway, and Buschinger, 1992, Heinze and Kauffmann, 1993
<i>Formicoxenus</i> spp.	guest-ant	<i>Myrmica</i> spp., <i>Formica</i> spp., <i>Manica mutica</i>	holarctic	Francoeur et al., 1985

L. cf. canadensis, the most common *Leptothorax* (s.str.) in New England, Québec and the Canadian Maritime Provinces is also referred to as "large black *L. 'muscorum'*" (e.g., Francoeur, 1986; Loiselle et al., 1990) or *Leptothorax* sp. B (e.g., Heinze and Buschinger, 1987, 1988; Heinze, 1989). However, the original description of *L. canadensis* (Provancher, 1887) fits quite nicely to this taxon. At present it is not known how far west *L. cf. canadensis* ranges, but morphologically, karyologically, and biochemically more or less similar ants (referred to as *Leptothorax "muscorum"* D and E, Heinze, 1989) occur throughout the Rocky Mountains and the Coast Mountains in western Canada and the western USA. *Leptothorax* sp. A is a widespread species in open coniferous forests and on partly shaded rocky patches in New England,

Québec, Ontario, and New Brunswick; a morphologically similar species, though with a different chromosome number, *Leptothorax "muscorum"* C, perhaps identical to the variety *L. muscorum septentrionalis* (Wheeler, 1917), is found in the Canadian Rocky Mountains.

Chromosomes were prepared from unpigmented male *Leptothorax* pupae following a procedure by Imai, Crozier, and Taylor 1977; (see also Loiselle, et al, 1990). For electrophoresis in 12.5 cm long 7.5% polyacrylamide gels, adults or pupae were crushed individually in 40 μ l of PAGE-homogenization buffer (0.1M Tris/HCl pH 8.0, 1mM EDTA, 0.05mM NADP, 2mM β -Mercaptoethanol, 10% glycerine, 0.01% bromothymol blue), of which 5 to 10 μ l were applied to the gel (gel buffer: 0.125M Tris/HCl pH 8.0; tray buffer 0.16M glycine, 0.025 M Tris, pH 8.3). Proteins were separated at 10°C with 10mA per gel for 1.5 hours. For detection of IDH, 0.25M Tris/HCl pH 9.6 was used both in the gel and as tray buffer. For electrophoresis on cellulose acetate plates (Titan III, Helena Laboratories, Beaumont, Texas), whole ants were crushed in 5-8 μ l tray buffer (with 0.01% bromothymol blue and amaranth as tracking dyes) and applied to the surface of the pre-soaked gel (tray buffer: 0.1M Tris/0.1M Maleat/0.01M EDTA, pH 7.4, 1:10) using the Helena "Super Z" applicator. Gels were run for 30 min. at 200 V at 5°C (see also Heinze, 1991). Of 14 to 20 enzymes screened in *Leptothorax acervorum*, *L. cf. canadensis*, and *L. sp. A*, 7 which could reliably be stained and showed a reasonable amount of inter- or intraspecific variation, were chosen for a more detailed analysis of both host species and parasites. In most non-parasitic species, at least 20 workers from 10 different colonies were stained for each enzyme; sample sizes are typically much larger in GPI and PGM. Fewer workers were available from *L. sphagnicolus*, *L. retractus*, and the parasitic taxa. In species with limited material, where no allozyme differences were found between populations (*L. paraxenus*, *L. wilsoni*, *F. quebecensis*), data from different populations were pooled for the analysis. Not all of those enzymes found to be invariable among the non-parasitic species were stained in the workerless parasites. Nei's indices were calculated from allele frequencies and clustered (UPGMA) using the computer program NTSYS (Rohlf, 1990); in addition, a neighbor-joining tree (Saitou and Nei, 1987) was calculated. Stability of clusters in the UPGMA phenogram was tested by

jackknifing over taxa (Lanyon, 1985) and by calculating a cophenetic regression coefficient.

Voucher specimens, wherever available, of the studied species are deposited in the MCZ, Cambridge, Mass., (USA).

RESULTS

Morphology

The current taxonomic confusion concerning the nearctic representatives of *Leptothorax* (s.str.) (e.g., Creighton, 1950; Brown, 1955; Francoeur et al., 1985; Heinze, 1989) makes a thorough morphological comparison between non-parasitic *Leptothorax* (s.str.) and the associated parasitic taxa difficult. Only few characters appear to be stable enough to serve for species distinction (Table 2). The most reliable and most frequently cited characters are the suberect hairs on tibia and scapes in *L. acervorum*, *L. sphagnicolus*, *Doronomyrmex*, and *Harpagoxenus*, and the indented clypeus in *L. gredleri*, *L. retractus*, *L. paraxenus*, and to a lesser extent also *Leptothorax* sp. A (Table 2). Both characters, however, may vary between populations: *L. acervorum* from Alaska, for example, are considerably less hairy than central European specimens (Heinze and Ortius, 1991). Other characters which serve to distinguish parasitic genera from *Leptothorax* (s. str.) are probably mostly adaptations to the parasites' specialized way of life and cannot be used to investigate phylogenetic relationships. *Harpagoxenus*, e.g., is easily recognized by strong, toothless mandibles and an extraordinarily large head with antennal scrobes, all obvious adaptations to slave-making. Similarly, the morphological features separating *Doronomyrmex* from *Leptothorax* are thought to be adaptations to the parasitic life, and some authors question whether *Doronomyrmex* should be kept as a distinct genus (Brown, 1973; Bolton, 1982).

In a detailed morphological revision, Francoeur et al. (1985) provided clear evidence for the separation of the guest-ant genus *Formicoxenus* from non-parasitic *Leptothorax* (s.str.). Female *Formicoxenus* are more slender than *Leptothorax* and the scape of *Formicoxenus* males is longer and more cylindrical than in *Leptothorax* males. Furthermore, whereas the majority of Formicoxenini, including non-parasitic *Leptothorax* and *Harpagoxenus*,

Table 2. Morphological characteristics and chromosome numbers of *Leptothorax* (s.str.) and their parasites, based on Bolton (1982), Francoeur et al. (1985), Loiselle et al. (1990), and own observations.

	Eyes with hairs	Erect hairs on legs	Enlarged Dufour's gland	Clypeus indented	Palp formula	Chromosomes
<i>L. acervorum</i>	-	+	-	-	5,3	13
<i>L. cf. canadensis</i>	-	-	-	-	5,3	17, 18
<i>L. gredleri</i>	-	-	+	+	5,3	11
<i>L. muscorum</i>	-	-	-	-	5,3	17, 18
<i>L. retractus</i>	-	-	-	+	5,3	17, 18
<i>L. sphagnicolus</i>	-	+	?	-	5,3	13
<i>L. sp. A</i>	-	-	+	-	5,3	14, 15, 16
<i>L. C</i>	?	-	?	-	5,3	18
<i>D. pocahontas</i>	?	+/-	?	-	5,3	18
<i>L. faberi</i>	?	-	?	+	5,3	15
<i>L. paraxenus</i>	-	-	+	+	5,3	15
<i>L. wilsoni</i>	+	-	+	-	4,3	?
<i>D. goesswaldi</i>	-	+	+	+		26, 28
<i>D. kutteri</i>	-	+	+	-	5,3	23, 25
<i>D. pacis</i>	-	+	+	-	4,3 and 5,3	26, 27
<i>H. canadensis</i>	-	+	+	±	5,3	18
<i>H. sublaevis</i>	-	+	+	-	5,3	20
<i>Formicoxenus</i> spp.	+	-	?	-	4,3 or 5,3	11-15

have a standard palp formula of 5 maxillary palps and 3 labial palps, the number may be reduced to 4, 3 in *Formicoxenus* (Bolton, 1982). However, as shown by Francoeur et al. (1985), there is intergeneric and even intraspecific variation in palp formula in *Formicoxenus*. The palp formula is similarly reduced to 4, 3 in *Leptothorax wilsoni* (two queens from Jasper N.P., Alberta, and one individual from Escoumins, Québec—the palp formula given in Heinze, 1989 for the type material needs to be confirmed by re-examination of material from the type locality) and *Doronomyrmex pacis* (two individuals from Jenner, Germany; according to Kutter, 1950 males of *D. pacis* from Switzerland have a palp formula of 5, 4). Palp formula is therefore probably not very informative on genus level (Table 2).

Formicoxenus have scattered hairs on their compound eyes, whereas the eyes are thought to be hairless in *Leptothorax* (Francoeur et al., 1985). Hairy eyes, however, are found in *L. wilsoni* (Heinze, 1989; Table 2). Despite the superficial similarity between *L. wilsoni* and *Formicoxenus* in these two characters, *L. wilsoni* is nevertheless morphologically closer to *Leptothorax* in others, such as the scape length in males. *L. wilsoni* shares reduced mandibular dentition with *Epimyrma*, a slave-making satellite genus of *Leptothorax* (*Myrafant*), but clearly differs in palp formula and the shapes of petiole and postpetiole.

Most queens of workerless parasites in Formicoxenini, and perhaps of workerless parasitic ants in general, are extraordinarily small compared to queens of the host species (Douwes, 1990; Nonacs and Tobin, 1992). Queens of *L. paraxenus* are a notable exception, in that they are of similar size as the host queens. As small size is thought to be adaptive in parasites—parasite queens do not need much resources for colony founding and thus, a larger number of less well equipped, small queens can be produced from the same amount of energy available to the colony (Douwes, 1990)—the condition in *L. paraxenus* might reflect a recent origin from a non-parasitic ancestor.

Parasite queens are typically characterized by a broadened postpetiole, a strong ventral petiolar spine, and a larger Dufour's gland compared to their hosts. These features, however, are not restricted to social parasites but may be found to a varying degree also in the queens of free-living species such as *Leptothorax* sp. A and *L. gredleri*.

Chromosome Number and Allozyme Phenotype

The significance of chromosome number as a taxonomic character is very poorly understood. Nevertheless, various studies have used chromosome analyses to clarify the taxonomy of Formicoxenini (Fischer, 1987; Heinze and Buschinger, 1989; Loiselle et al., 1990; Buschinger and Fischer, 1991). Chromosome numbers in *Leptothorax* (s.str.) and associated genera range between 11 and appr. 28. Palearctic *Doronomyrmex* have much higher chromosome numbers than their common host, *L. acervorum*. In contrast, the nearctic workerless parasites *L. faberi* (Buschinger, 1982) and *L. paraxenus* both have 15 chromosomes (*L. paraxenus*: 30 metaphase plates from 4 male pupae from Milton, Ontario),

whereas their host *L. cf. canadensis* has 17 or 18 (Heinze and Buschinger, 1989; Loiselle et al., 1990).

UPGMA clustering of Nei's indices calculated from seven enzyme systems which are of diagnostic value in the studied species results in the phenogram shown in Fig. 1. Original data and Nei's indices are given in Tables 3 and 4. Goodness of fit of the cluster analysis to the data was tested by comparing a matrix of cophenetic values, calculated from the tree matrix, with the original similarity matrix. The resulting cophenetic correlation of $r = 0.809$ suggests a rather mediocre fit (Mantel t-test, $t = 6.53$, $p = 1.000$). Nevertheless, the overall branching pattern is remarkably stable. Two fundamental dichotomies are found in all 21

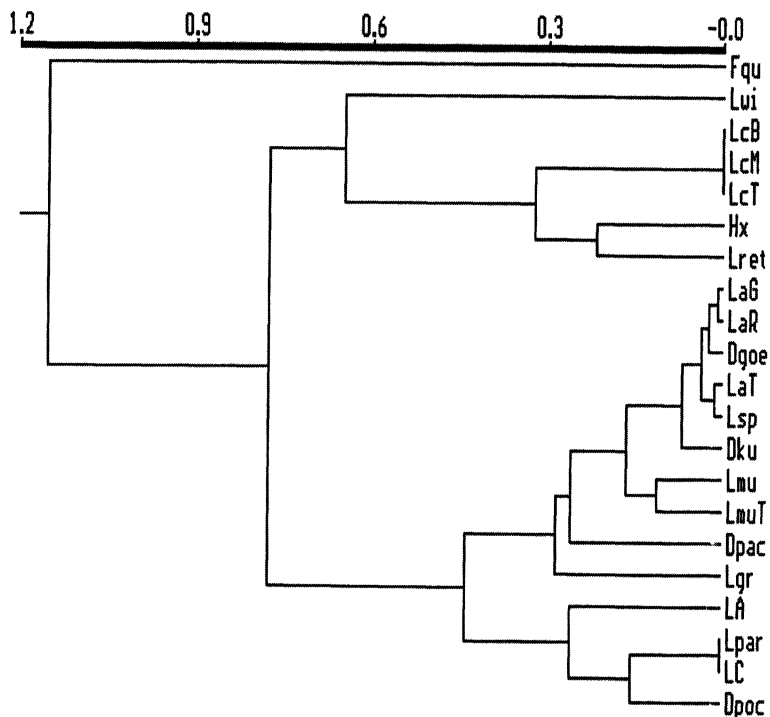


Fig. 1. Phenogram obtained by UPGMA clustering of Nei's indices from electromorph frequencies of several species of *Leptothorax* (s.str.), *Doronomyrmex*, *Formicoxenus quebecensis*, and *Harpagoxenus sublaevis*. For abbreviations see Table 3.

pseudoreplicates generated from the original data set by jackknifing over taxa. Firstly, *Formicoxenus*, represented in the electrophoretic study by *F. quebecensis*, is least similar to the other taxa, and secondly, a cluster consisting of *Harpagoxenus sublaevis*, *L. retractus*, *L. cf. canadensis*, and *L. wilsoni* (cluster A), stands in sharp contrast to the remaining taxa (*L. acervorum*, *L. sphagnicolus*, *L. muscorum*, *L. gredleri*, *L. paraxenus* and *Doronomyrmex*, cluster B). *L. acervorum*, *L. sphagnicolus* and the two workerless parasites, *D. kutteri* and *D. goesswaldi*, also form a stable group (supported in all pseudoreplicates), which is probably reflected in morphological similarities among these species, such as the presence of erect hairs on the scapes and legs. Though *Harpagoxenus* and *D. pacis* likewise share this character, at least *Harpagoxenus* appears biochemically quite dissimilar from the *L. acervorum* group. The branching pattern in the lower half of cluster B (*L. muscorum*, *L. gredleri*, *L. sp. A*, *L. paraxenus*, *D. pacis*, and *D. pocahontas*) was present in only 19 of 21 replicates and thus is less well supported by the data. It therefore would be premature to conclude that *D. pacis* is not very close to its workerless congeners

Table 3. Frequency of electromorphs of 7 enzymes in the ant genus *Leptothorax* (s.str.) and associated parasitic taxa. The abbreviations stand for the following species: *Dgoe*: *D. goesswaldi* (9 virgin queens from 3 colonies); *Dku*: *D. kutteri* (8 virgin queens from 2 colonies); *Dpac*: *D. pacis* (5 virgin queens from 3 colonies); *Dpoc*: *D. pocahontas* (10 workers from 3 colonies); *Fqu*: *Formicoxenus quebecensis* (12 workers from 4 colonies from different populations); *Hx*: *Harpagoxenus sublaevis* (26 workers from 8 colonies); *LA*: *L. sp. A* (209 workers from 17 colonies); *LaG*, *LaR*, *LaT*: *L. acervorum* from Großostheim (Germany, 196 workers from 19 colonies), Reichswald (Germany, between 64 and 1420 workers from 10 to 140 colonies), and Ilgaz Dagı Geçidi (Turkey, 49 workers from 7 colonies); *LC*: *Leptothorax* C (10 workers from 4 colonies); *LcB*, *LcM*, *LcT*: *L. cf. canadensis* from Bic (Québec, 113 workers from 13 colonies), Mt. Monadnock (New Hampshire, 131 workers from 17 colonies) and Tadoussac (Québec, 66 workers from 10 colonies); *Lgr*: *L. gredleri* (between 20 and 299 workers from 29 colonies); *Lmu*, *LmuT*: *L. muscorum* from Reichswald (between 20 and 569 workers from 76 colonies) and Ilgaz Dagı Geçidi (20 workers from 5 colonies); *Lpar*: *L. paraxenus* (10 virgin queens from 4 colonies); *Lret*: *L. retractus* (10 workers from 2 colonies); *Lsp*: *L. sphagnicolus* (10 workers from 2 colonies); *Lwi*: *L. wilsoni* (8 virgin queens from 4 colonies).

The enzymes are Glucose-6-phosphate isomerase (GPI, referred to as PGI in Heinze, 1989, 1991), Phosphoglucumutase (PGM), 6-Phosphoglucuronate dehydrogenase (PGD), Malate dehydrogenase (MDH), Isocitrate dehydrogenase (IDH), Malic enzyme (ME), and Lactate dehydrogenase (LDH). All species share the same electromorph of tetrazolium oxidase. v, s, m, n, f, and x denote different migration velocity during electrophoresis; blanks in the table indicate missing data.

<i>Dgoe</i>	<i>Dku</i>	<i>Dpac</i>	<i>Dpoc</i>	<i>Fqu</i>	<i>Hx</i>	<i>LA</i>	<i>LaG</i>	<i>LaR</i>	<i>LaT</i>	<i>LC</i>	<i>LcB</i>	<i>LcM</i>	<i>LcT</i>	<i>Lgr</i>	<i>Lmu</i>	<i>LmuT</i>	<i>Lpar</i>	<i>Lret</i>	<i>Lsp</i>	<i>Lwi</i>
0.00	0.00	1.00	0.00	1.00	0.00	0.49	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00
0.00	0.00	0.00	1.00	0.00	1.00	0.51	0.12	0.06	0.00	1.00	0.02	0.02	0.02	0.98	0.95	1.00	1.00	0.00	0.00	1.00
0.50	1.00	0.00	0.00	0.00	0.00	0.00	0.69	0.82	0.88	0.00	0.93	0.90	0.95	0.01	0.04	0.00	0.00	1.00	1.00	0.00
0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.11	0.12	0.00	0.05	0.07	0.04	0.01	0.00	0.00	0.00	0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00		0.00	0.00	0.00	0.00	0.04	0.01	0.00	0.00	0.05	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	1.00
	1.00		0.00	1.00	0.50	0.00	0.55	0.42	0.31	0.00	0.04	0.06	0.12	0.31	0.01	1.00	0.00	1.00	0.00	0.00
	0.00		1.00	0.00	0.50	0.95	0.24	0.42	0.69	1.00	0.89	0.86	0.85	0.69	0.78	0.00	1.00	0.00	1.00	0.00
	0.00		0.00	0.00	0.00	0.05	0.17	0.15	0.00	0.00	0.02	0.08	0.01	0.00	0.21	0.00	0.00	0.00	0.00	0.00
1.00	1.00		0.83	0.00	0.00	0.84	0.92	0.79	1.00	1.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00	1.00	0.00
0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
0.00	0.00		0.17	0.00	1.00	0.16	0.08	0.21	0.00	0.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.80	0.00	0.00
0.00	0.00		0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.00	1.00	1.00	1.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
0.00	0.00	0.00	0.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
1.00	1.00		0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
0.00	0.00		1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00
				1.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00
					1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00

Table 4. Nei's indices calculated from electromorph frequencies in Table 3.

	<i>Dgoe</i>	<i>Dku</i>	<i>Dpac</i>	<i>Dpoc</i>	<i>Fqu</i>	<i>Hx</i>	<i>LA</i>	<i>LaG</i>	<i>LaR</i>	<i>LaT</i>	<i>LC</i>	<i>LcB</i>	<i>LcM</i>	<i>LcT</i>	<i>Lgr</i>	<i>Lmu</i>	<i>LmuT</i>	<i>Lpar</i>	<i>Lret</i>	<i>Lsp</i>	<i>Lwi</i>
<i>Dgoe</i>	0.000																				
<i>Dku</i>	0.044	0.000																			
<i>Dpac</i>	0.221	0.288	0.000																		
<i>Dpoc</i>	0.381	0.582	0.288	0.000																	
<i>Fqu</i>	1.055	0.847	0.693	1.925	0.000																
<i>Hx</i>	0.650	0.656	0.693	0.589	0.794	0.000															
<i>LA</i>	0.636	0.836	0.405	0.215	1.108	0.353	0.000														
<i>LaG</i>	0.018	0.030	0.223	0.407	1.059	0.437	0.531	0.000													
<i>LaR</i>	0.032	0.048	0.246	0.399	1.107	0.418	0.517	0.008	0.000												
<i>LaT</i>	0.025	0.076	0.260	0.368	1.201	0.501	0.477	0.025	0.013	0.000											
<i>LC</i>	0.362	0.560	0.288	0.004	1.946	0.656	0.213	0.394	0.398	0.352	1.270	0.000									
<i>LcB</i>	0.826	0.834	1.370	1.166	0.947	0.323	0.610	0.595	0.498	0.523	1.279	0.001	0.000								
<i>LcM</i>	0.823	0.830	1.349	1.174	0.929	0.317	0.608	0.590	0.496	0.525	1.279	0.001	0.000	0.000							
<i>LcT</i>	0.827	0.798	1.373	1.189	0.917	0.318	0.619	0.582	0.490	0.519	1.294	0.001	0.001	0.000	0.000						
<i>Lgr</i>	0.357	0.449	0.279	0.350	1.208	0.316	0.568	0.267	0.262	0.288	0.370	0.730	0.727	0.733	0.000						
<i>Lmu</i>	0.127	0.294	0.272	0.176	1.348	0.342	0.369	0.127	0.134	0.125	0.167	0.705	0.701	0.713	0.153	0.000					
<i>LmuT</i>	0.139	0.154	0.288	0.350	0.981	0.342	0.553	0.109	0.157	0.196	0.336	0.940	0.925	0.912	0.211	0.111	0.000				
<i>Lpar</i>	0.362	0.560	0.288	0.004	2.079	0.543	0.183	0.331	0.332	0.299	0.000	0.987	0.991	1.002	0.314	0.144	0.288	0.000			
<i>Lret</i>	0.468	0.313	0.693	1.145	0.487	0.218	0.716	0.365	0.339	0.443	1.229	0.337	0.331	0.313	0.689	0.775	0.536	1.229	0.000		
<i>Lsp</i>	0.044	0.154	0.288	0.350	1.386	0.543	0.456	0.071	0.043	0.013	0.336	0.484	0.491	0.488	0.308	0.131	0.288	0.288	0.536	0.000	
<i>Lwi</i>	1.055	1.253	1.386	1.232	0.981	0.438	0.768	0.843	0.872	0.939	1.253	0.655	0.659	0.661	0.669	0.677	0.693	0.981	0.824	0.981	0.000

and *L. acervorum*, as suggested by the phenogram in Fig. 1. It has recently been shown that *D. pocahontas* is not a workerless parasite and thus does probably not belong to the workerless *Doronomyrmex*. The shiny, long-haired queens, morphologically similar to *D. pacis* but strikingly different from their nestmate workers, are probably a special morph of a species whose queens and workers are typically short-haired and dull (Buschinger and Heinze, 1993). In a previous study using cellulose acetate electrophoresis, "shiny" queens and workers of *D. pocahontas* from three colonies from the type locality differed from other studied species in having a very slowly migrating electromorph in the enzyme 6-Phosphogluconate dehydrogenase (PGD, Heinze 1989). According to the results of the present study with polyacrylamide gels, workers from three colonies with "dull" queens from the same collecting site, however, predominantly had the slowly migrating PGD electromorph found commonly also in other taxa in cluster B (Table 3). In one colony, workers were heterozygous for the slow and the fast migrating electromorph. More data on this ant and morphologically similar taxa from the Rocky Mountains are needed to elucidate the status of *D. pocahontas*.

L. paraxenus differs from its host, *L. cf. canadensis* in several enzymes (Table 3) and is attached to cluster B. *L. wilsoni* is closely associated neither with one of its two hosts, *L. cf. canadensis* and *L. sp. A*, nor with *Formicoxenus*, but is more similar to species in cluster A than to cluster B species.

In a neighbor joining tree, which, however, showed poorer fit to the original data ($r = 0.691$, $t = 5.174$, $p = 1.000$), *F. quebecensis* is attached to *L. retractus* and *L. cf. canadensis*, whereas *L. wilsoni* and *Harpagoxenus* are closer to the taxa of cluster B.

DISCUSSION

Morphology, allozyme phenotype, and chromosome number were compared to analyze the evolution of workerless social parasites in the ant tribe Formicoxenini. Though the data are as yet not sufficient for a thorough cladistic analysis, similarities in morphological and biochemical characters which I consider to be neutral in natural selection support the results of previous more qualitative studies (Buschinger, 1981, 1990; Heinze, 1991).

The slave-making and workerless parasites studied here appear to be phylogenetically close to their hosts. Though the workerless *L. wilsoni* and the guest-ant genus *Formicoxenus* differ in several enzyme systems and morphological features from other taxa investigated in this study, they clearly belong to the Formicoxenini and within this tribe are probably closer to *Leptothorax* (s.str.) and its parasites than to *Leptothorax* (*Myrafant*) or other *Leptothorax* subgenera (see also Buschinger, 1981; Francoeur et al., 1985; Heinze, 1991). *Harpagoxenus* slave-makers and workerless *Leptothorax* and *Doronomyrmex* clearly cluster with species of *Leptothorax* (s.str). The data thus support what has been called "a loose version of Emery's rule." In 1909, Emery suggested for slave-makers and workerless parasites that they "all originate from closely related forms which serve them as slave or host species," based on morphological similarities between parasites and hosts. Ward (1989) proposed to distinguish between a loose form of Emery's rule—parasites and their hosts are close relatives (see also Wasmann, 1909)—and a strict form—for a given parasite-host pair, the sister group of the parasite lineage includes the host species (Ward, pers. comm.). The loose version appears to hold in all host-parasite pairs investigated so far (Ward, 1989; Agosti, 1994; Sanetra, Heinze and Buschinger, 1994), with exception of guest ants which explicitly were never included in this rule. A relationship as suggested by the strict version, resulting from sympatric or allopatric speciation, cannot be disproven for some formicoxenine ants. Allozyme similarities suggest a close relationship between at least two of the three palaearctic, workerless *Doronomyrmex* and their common host, *L. acervorum*, as previously deduced from morphological characters (e.g., the presence of suberect hairs on scapes and tibia (Buschinger, 1990)), sequence comparisons of a mitochondrial cytochrome b gene (Baur, Sanetra, Chalwatzis, Buschinger and Zimmermann, 1995), and an internal transcribed spacer adjacent to the 5.8S rRNA gene (Baur, Sanetra, Chalwatzis, Buschinger and Zimmermann, 1996). On the other hand, *L. acervorum*, *L. gredleri*, *L. muscorum*, and other taxa in cluster B are very similar in almost all studied enzyme systems, and it therefore cannot be ruled out that the three *Doronomyrmex* originated jointly or independently from ancestors other than *L. acervorum*.

Morphological similarities between the *Doronomyrmex* species and *L. acervorum* might be convergent adaptations to parasitic life with a common host species instead of synapomorphies. Morphological traits of parasites might serve to "camouflage" the parasite in the host colony or to increase the parasite queen's colony founding success. Hair length, e.g., may vary within a monophyletic group, perhaps in response to the condition in the host: the degenerate slave-maker *Epimyrma kraussei* has long hairs like its host, *Leptothorax recedens*, but much other data suggests a common origin of long- and short-haired *Epimyrma* (Buschinger, 1989). In any case, the result that the workerless *Doronomyrmex* are closer to some *Leptothorax* (s.str.) (cluster B) than to others (cluster A) clearly corroborates the view that the genus *Doronomyrmex* ought to be synonymized under *Leptothorax* (s.str.) (Brown, 1973; Bolton, 1982). Alternatively, *Leptothorax* (s.str.) would be a paraphyletic taxon. I therefore propose to synonymize *Doronomyrmex* under *Leptothorax* (s.str.).

The strict version of Emery's rule is not supported in *L. paraxenus* and *L. wilsoni*. Both parasites are clearly distinct in morphology, karyotype, and enzyme pattern from their common host, *L. cf. canadensis*. *L. wilsoni* was recently found in colonies of a second host species, *Leptothorax* sp. A (Heinze et al., 1995), but a closer phylogenetic relationship between them also appears unlikely based on morphology and enzyme patterns.

Buschinger provided quite firm evidence that in the formicoxenine genera *Chalepoxenus* (Buschinger et al., 1988) and *Epimyrma* (Buschinger, 1989) workerless parasites evolved from non-parasitic ancestors via slave-makers, but rejected this evolutionary pathway for *Doronomyrmex* (Buschinger, 1990). The data presented in this study indeed do not support the assumption that workerless *Doronomyrmex* and *Leptothorax* are degenerate slave-makers or have evolved from guest-ants. *L. wilsoni* shows some morphological and biochemical similarities to *Formicoxenus* (e.g., hairy eyes and a reduced palp formula), but is more similar to *Leptothorax* (s.str.) in other morphological features; its current systematic position is unclear. From morphological similarities, high chromosome number, and intergeneric attractiveness of sexual pheromones, Buschinger (1990) concluded that *Harpagoxenus* and *Doronomyrmex* probably form a monophyletic group. The

supposed monophyly is not reflected in the phenogram based on enzyme similarities. Some of the morphological similarities (ventral postpetiolar spine, hypertrophied Dufour's gland, gray wing buds in sexual pupae) are probably convergent adaptations to the parasitic way of life. The ventral postpetiolar process, for example, is part of the "inquiline syndrome" (Wilson, 1971) and appears to be an almost universal characteristic of myrmicine parasites, including *Formicoxenus*, *L. wilsoni*, *L. paraxenus*, and parasitic ants in other tribes, such as *Myrmica*, and probably serves to increase the stability of the petiolus-postpetiolus-gaster region against attacks by host workers. Similarly, a hypertrophied Dufour's gland is a common feature in both parasitic and non-parasitic Formicoxenini in which queens fight, either during parasitic colony founding (Buschinger, 1974; Allies, Bourke and Franks, 1986) or during the establishment of reproductive hierarchies in functionally monogynous *Leptothorax* (Heinze and Smith, 1990; Heinze, Lipski and Hölldobler, 1992). According to recent observations, gray wing buds in sexual pupae are not a synapomorphy of *Doronomyrmex* and *Harpagoxenus*, but occasionally occur also in other species of both parasitic and non-parasitic *Leptothorax* (unpubl. observations: *L. muscorum*, *L. gredleri*, *L. paraxenus*). Intergeneric attraction of sexual pheromones between *Doronomyrmex pacis* and *Harpagoxenus* speaks in favor of a close relationship, however; whether the sex pheromones of other formicoxenine queens are less attractive to *Harpagoxenus* males still has to be tested. In an analysis by Baur et al. (1995, 1996), *Harpagoxenus* clearly clusters with *Leptothorax* (s.str.), but its position relative to *Doronomyrmex* and *L. acervorum* differs between neighbor-joining trees based on the sequences of cytochrome b and a spacer region of ribosomal genes.

A third hypothesis on the origin of workerless social parasites suggests that parasites evolved from one ancestor species, but parasitize another, less closely related species. According to enzyme phenotypes and chromosome numbers, the workerless social parasite *Leptothorax paraxenus* is more similar to *Leptothorax* sp. A and other *Leptothorax* in cluster B than to its host, *L. cf. canadensis*. It appears likely that *L. paraxenus* evolved from small brown *Leptothorax* ancestors and somehow started to parasitize the large black *Leptothorax cf. canadensis*. This possibility of host transfer

has previously been considered by West-Eberhard (1990) and Bourke and Franks (1991) to explain the occurrence of parasite-host pairs only loosely following Emery's rule: in a species with alternative reproductive strategies, a phenotype adapted to parasitizing conspecific colonies might have switched from its conspecific ancestral host to a new host species.

ACKNOWLEDGMENTS

This study was supported by the Deutsche Forschungsgemeinschaft (Heisenberg-grant He 1623/6-1). I thank S. Trenkle for technical assistance, S. Kauffmann, B. Hülsen, S.P. Cover, T.M. Alloway and others for help in the field, and A. Buschinger and R. Schumann for material of *L. wilsoni* and *Formicoxenus quebecensis* from populations in western North America. P. S. Ward, Univ. of California, Davis, provided helpful comments on the manuscript.

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Note added in the proof:

Further investigations since the submission of the manuscript have led to small changes in allele frequencies, which slightly affect the branching pattern in cluster B. The overall pattern, however, remained stable.