Z. zool. Syst. Evolut.-forsch. 29 (1991) 165–175
© 1991 Verlag Paul Parey, Hamburg und Berlin
ISSN 0044-3808

Received on 31. August 1990

Department of Zoology, University of Lund, Sweden

Hybridization and variation in the Leptothorax tuberum group (Hymenoptera: Formicidae)

By P. Douwes and B. Stille

Abstract

Morphological and allozymatic analyses show that there are four species – *L. tuberum* (mainly in the north) and *L. nigriceps*, *L. "tubero-interruptus"*, and *L. unifasciatus* (sympatrically in the south) – in the *Leptothorax tuberum* group in West Europe north of the Alps, and that these species hybridize. The two commonly co-occurring species, *L. nigriceps* and *L. unifasciatus*, rarely hybridize, suggesting that premating isolating mechanisms have evolved, whereas the rare species, *L. "tubero-interruptus"*, easily interbreeds possibly with all the other species of this group. There are distinct morphological gaps between the species, but hybridization tends to fill these gaps or even produces morphological copies (*L. nigriceps* × *L. "tubero-interruptus"*) of a third species (*L. tuberum*).

Key words: Quantitative variation – Allozymes – Hybridization

Introduction

Leptothorax Mayr, 1855 is a mainly holarctic myrmicine ant genus (Emery 1922) with at least 40 species in Europe (Collingwood 1979); Agosti (1989) lists 88 species from Europe. Although the systematics of this genus is confused, the identity and distribution of the species in Northwest and Central Europe appear to be well known (Collingwood 1979; Kutter 1977). In this area there is a group of morphologically similar species, the *L. tuberum* group, including the species *L. interruptus* (Schenck, 1852), *L. nigriceps* Mayr, 1855, *L. tuberum* (Fabricius, 1775), and *L. unifasciatus* (Latreille, 1798) (Kutter 1977). We exclude *L. interruptus* here since this species has a different chromosome number (n=12) than the other species (n=9) (Fischer 1987) and different alleles at two enzyme loci (Douwes and Stille 1987). Moreover, morphologically *L. interruptus* is characteristic enough not to be confused with any of the other species in the *L. tuberum* group. Apparently also belonging to this group is a species called *L. "tubero-interruptus"* (*Leptothorax tuberum* v. *tubero-interrupta* Forel, 1874 sensu Plateaux 1978), although it has a different chromosome number (n=8) (Fischer 1987). This species appears rarely in the literature and is not mentioned by Kutter (1977). The chromosome evidence given by Fischer (1987) shows that it is a distinct species and from Buschinger (pers. comm.), Douwes and Stille (1987); Fischer (pers. comm.), and Plateaux (l.c.) it is obvious that *L. "tubero-interruptus"* is similar to the *L. tuberum* group species.

After having collected these ants for many years, we have learned that the systematics of the North and Central European *L. tuberum* group species is far from clear. It is still a problem to discriminate between *L. tuberum* and *L. "tubero-interruptus"* (Seifert, pers. comm.) and between the latter and *L. unifasciatus* (own obs.), perhaps due to the fact that some *L. "tubero-interruptus"* are hybrids (Fischer 1987). *L. nigriceps* and *L. unifasciatus* also hybridize (Seifert 1984) and hybridization between *L. tuberum* group species might be more frequent than has been realized.

Using genetic markers (allozymes) would be a way to detect hybrids. In a previous study (Douwes and Stille 1987) we demonstrated interspecific allozyme variation in the *L. tuberum* group and using another buffer for PGI and MDH there is enough resolution to detect hybrids in most combinations of these species.

Material and methods

In 1984–1988 we collected 246 colonies of *L. tuberum* group species in 20 different localities (Fig. 1). The whole colony was collected (if possible) and some of the workers were stored at −70°C until electrophoresis. Additionally, B. Seifert provided us with a small, selected sample from loc. 21 (Fig. 1).

The electrophoretic and staining techniques used are as described by Selander et al. (1971). From each colony 1–10 (usually 2) ants were individually analyzed. Whole ants were homogenized in deionized water and four enzyme loci were stained: PGI (phosphoglucose isomerase), MDH-1 (anodal malate dehydrogenase), and MDH-2 (cathodal MDH) resolved on a Tris citrate buffer (gel pH 8.4/ bridge pH 7.1) (Varvio-Aho and Pamilo 1980) and PGM (phosphoglucomutase) on a Tris-maleic-EDTA buffer (gel pH 7.4/ bridge pH 7.4). The allozymes were denoted with letters according to relative migration (F = fast, M = medium, S = slow). The variation in PGM, however, could not be understood in terms of allelic variation (Douwes and Stille 1987) and we have therefore simply recorded the phenotypes obtained (1 to 6, 6 is fastest), each phenotype normally consisting of a strong and a weak band.

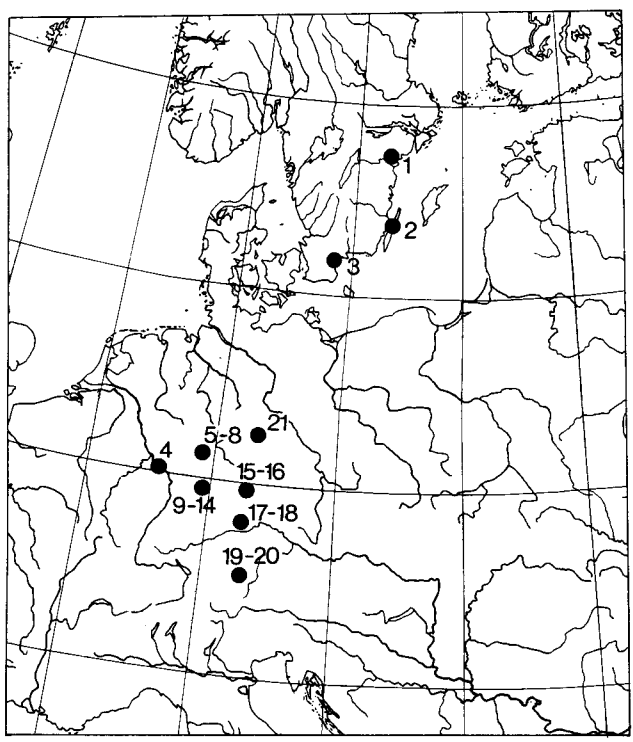

Fig. 1. Localities 1–21 in Sweden and Germany where *Lepthothorax tuberum* group species were collected

The *L. tuberum* group species are discriminated by different degrees of melanism on various body parts and to some extent also by sculpture (Kutter 1977). In our morphological analysis on five workers from each colony we have scored the following four characters: melanism of head (1 to 3), of 3rd leg femur (0 to 2) and of the first gaster tergite that varies from almost totally dark to mainly yellowish with a dark band. We recorded the length of the dark area (along the median line) relative to tergite length (0 to 3) and whether this area is a distinct band (score = 2, as in most *L. unifasciatus*), not well demarcated (0, as in e. g. *L. tuberum*) or midway between these extremes (1, as in most

L. "tubero-interruptus"). Moreover, the alitrunk length in queens (not available for all colonies) was measured.

The relationships between the colonies based on the samples of five workers and the morphological characters mentioned above were analyzed by principal components and after having included the information from the electrophoresis by a discriminant analysis (NORUSIS 1966).

Results

The principal components analysis reveals a high correlation between all morphological characters; the first axis represents 79 %, the second 12 % and hence together 91 % of the variation. This means that the morphological affinities of our 246 samples can be accurately represented by two dimensions (Fig. 2). All four characters are strongly correlated with the first axis and a high value along that axis means dark head, darkened femur, and extensive but weakly demarcated dark area on first gaster tergite. The second axis, being rather closely related to two characters, gives large values to samples with darkened femur and distinct gaster band. Excluding some outliers two clusters are shown, the upper one to the right corresponding to *L. nigriceps* and a large heterogeneous group of which top-left corresponds to *L. unifasciatus*, bottom-left to *L. "tubero-interruptus"* and bottom-right to *L. tuberum*. The three samples between *L. unifasciatus* and *L. nigriceps* have characteristics from both these species.

The variation at the PGI, MDH-1 and PGM loci based on mean allele frequencies for each sample is depicted in Figs. 3–6. As in the morphological variation species seem to be

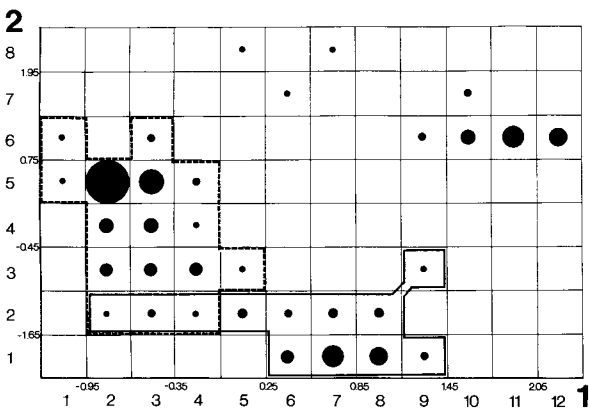

Fig. 2. Principal components analysis of 246 colonies of the *Leptothorax tuberum* group (morphological characters in workers, see text). The two first (1 and 2) axes, representing 91 % of the variation, are shown. The area of the solid circles is approx. proportional to the number of colonies (the smallest = 1 colony, the largest = 82 colonies). The colonies enclosed by the dashed line are analysed in Table 1, and those enclosed by the solid line in Table 2

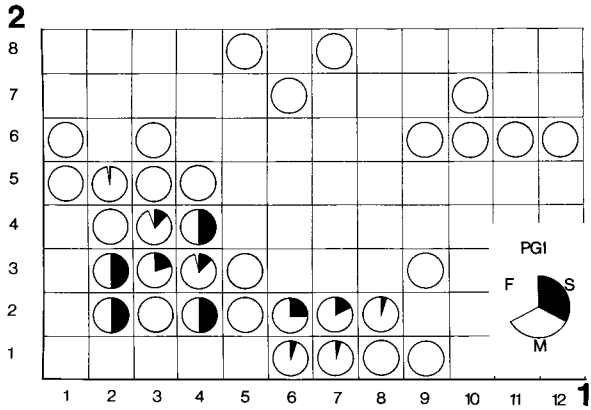

Fig. 3. The variation at the PGI locus (3 alleles) calculated from colony mean allele frequencies (cf. Fig. 2)

poorly defined by allozymes, although three clusters are indicated by PGM (*L. "tubero-interruptus"*), MDH-1 (*L. nigriceps*) and MDH-2 (*L. tuberum*). A closer examination of the covariation in morphology and allozymes will, however, illuminate the situation substantially.

The left hand side of the cluster in Fig. 2 encircled by the dashed line includes the samples thought to be *L. unifasciatus* and *L. "tubero-interruptus"*. There is a more or less continuous morphological variation along axis 2 from typical *L. "tubero-interruptus"* (class 2) to typical *L. unifasciatus* (classes 5–6). PGM has a bimodal variation along this axis so that a large peak (around electromorph 3) is associated with the *L. unifasciatus* morphotype and a small peak (electromorphs 5–6) is associated with the *L. "tubero-interruptus"* morphotype (Table 1). Furthermore, there is an association between PGM 5–6 and the S allele in PGI and between PGM 1–3.9 and PGI F, MDH-1 S, and MDH-1 F. Thus we have *L. unifasciatus* (PGM 1–3.9, PGI M, MDH-1 S, M, F) in classes 3–6 and *"tubero-interruptus"* (PGM 5–6, PGM S, M, MDH-1 M) in classes 2–4 (see also Table 3). this leaves us with seven samples that are intermediate in the electromorph as well as the morphological variation (classes 3–4). The most likely interpretation is that they are hybrids, especially since all but one are from locality 7 where both parental species were abundant (Table 6). Queens of *L. unifasciatus* are large (alitrunk length $\bar{x}$ = 1.32 mm, SD = 0.06, n = 86) while those of *L. "tubero-interruptus"* are small ($\bar{x}$ = 1.15, SD = 0.02, n = 7). Queens of the hybrid colonies are either small (1.14, 1.14, 1.15) or large (1.34) suggesting that both sexes in both species are involved in hybridization, or that not all colonies were F_1 hybrids.

Table 1. **Variation at the PGM, PGI and MDH-1 loci in the Leptothorax unifasciatus – "tubero-interruptus" complex**

Based on this variation colonies are assigned to *L. "tubero-interruptus"*(TUIN, bold figures), *L. "tubero-interruptus"*×*unifasciatus* (TUIN×UNIF, italics) or *L. unifasciatus* (UNIF, standard). PC2 = second principal component axis (see Fig. 2)

PC2	Number of colonies PGM phenotype					PGI-S	PGI F, MDH-1 S and/or MDH-1 F	"Species"
	1–1.9	2–2.9	3–3.9	4–4.9	5–5.9			
2					**2**	p>0	p=0	
					2	p=0	p=0	
3			*1*	*3*	**7**	p>0	p=0	
			3	*1*	**5**	p=0	p=0	
		1	1			p=0	p>0	
4					1	p>0	p=0	
			1			p>0	p>0	
			7			p=0	p=0	
		1	*7*	*1*		p=0	p>0	
5–6	8	16	26			p=0	p=0	
	3	8	54			p=0	p>0	
Totals					**10**	p>0	p=0	TUIN
					7	p=0	p=0	TUIN
				3		p>0	p=0	TUIN×UNIF
				1		p=0	p=0	TUIN×UNIF
				1		p=0	p>0	TUIN×UNIF
			1			p>0	p=0	TUIN×UNIF
			1			p>0	p>0	TUIN×UNIF
			36			p=0	p=0	UNIF
			62			p=0	p>0	UNIF
		16				p=0	p=0	UNIF
		10				p=0	p>0	UNIF
	8					p=0	p=0	UNIF
	3					p=0	p>0	UNIF

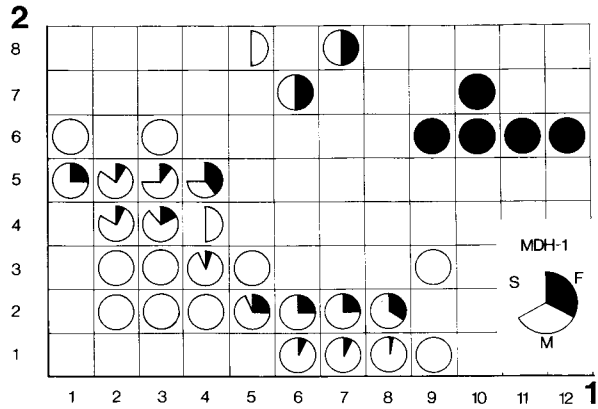

Fig. 4. The variation at the MDH-1 locus (3 alleles) calculated from colony mean allele frequencies (cf. Fig. 2)

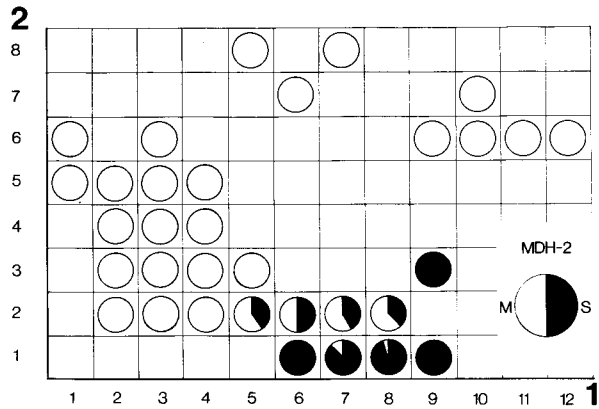

Fig. 5. The variation at the MDH-2 locus (2 alleles) calculated from colony mean allele frequencies (cf. Fig. 2)

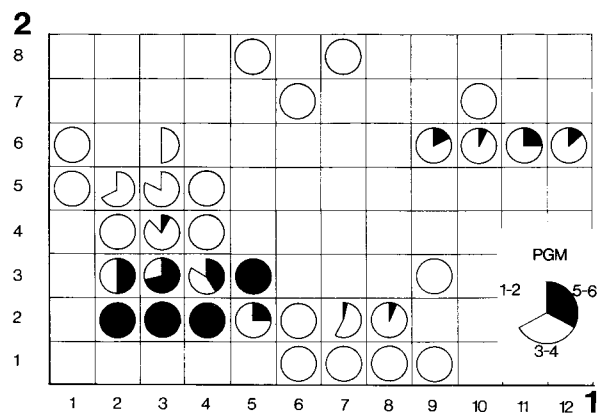

Fig. 6. The variation at the PGM locus (6 phenotypes) calculated from colony mean phenotype frequencies (cf. Fig. 2)

Another case of gradual morphological change between two species is that between *L. "tubero-interruptus"* and *L. tuberum*, enclosed by the solid line in Fig. 2. All four colonies in classes 2–4 along axis 1 (*L. "tubero-interruptus"*) have the same and unique (in this comparison) genotype (MDH-1 MM, MDH-2 MM; Table 2). Among the rest (classes 5–9) that more or less corresponds to *L. tuberum* all samples from Sweden and two from loc. 21 are monomorphic for the MDH-2 F allele (Table 2). This is *L. tuberum* (type locality = Sweden, Fabricius 1775) which, with the exception of two records of *L. unifasciatus*, is the only species in the *L. tuberum* group recorded from Sweden (COLLINGWOOD 1979).

Table 2. **Variation at the MDH-1, MDH-2, and PGM loci in the Leptothorax "tubero-interruptus" – tuberum complex**

Based on this variation colonies are assigned to *L. "tubero-interruptus"*(TUIN), *L. tuberum* (TUBE), *L. "tubero-interruptus"×tuberum* (TUIN×TUBE), or *L. nigriceps×"tubero-interruptus"* (NIGR×TUIN). PC1 = first principal components axis (see Fig. 2)

PC1	Number of colonies				PGM phenotype	Locality	"Species"
	MDH-1 MDH-2 MM	MM MM	FM	FF	FM MM			
2–4		4				5–5.9	7	TUIN
5			1			3–3.9	21	TUIN×TUBE
				1		3–3.9	1	TUBE
					1	3–3.9	7	TUIN×NIGR
					1	5–5.9	7	TUIN×NIGR
6				7		3–3.9	1, 2	TUBE
					1	3–3.9	7	TUIN×NIGR
					1	4–4.9	7	TUIN×NIGR
7			1			3–3.9	21	TUIN×TUBE
				1		2–2.9	1	TUBE
				10		3–3.9	1, 2, 20, 21	TUBE
					3	3–3.9	7	TUIN×NIGR
					1	4–4.9	7	TUIN×NIGR
8				10		3–3.9	1–3, 21	TUBE
				1		4–4.9	1	TUBE
					2	3–3.9	7	TUIN×NIGR
					1	4–4.9	7	TUIN×NIGR
9				2		3–3.9	1, 3	TUBE
				1		4–4.9	3	TUBE
Totals		4				5–5.9		TUIN
			2			3–3.9		TUIN×TUBE
				1		2–2.9		TUBE
				30		3–3.9		TUBE
				2		4–4.9		TUBE
					7	3–3.9		TUIN×NIGR
					3	4–4.9		TUIN×NIGR
					1	5–5.9		TUIN×NIGR

Table 3. **Allele and phenotype (PGM) frequencies calculated from colony means of Leptothorax tuberum group species and hybrids**

(For abbreviations of species names see Tables 1–2)

"Species"	Frequency (%)													
	PGI			MDH-1			MDH-2		PGM					
	F	M	S	F	M	S	F	M	1	2	3	4	5	6
UNIF	2	98	–	18	73	9	–	100	6	19	74	1	–	–
UNIF×TUIN	–	55	45	7	86	7	–	100	–	–	21	57	21	–
TUIN	–	71	29	–	100	–	–	100	–	–	–	–	97	3
UNIF×NIGR	–	100	–	50	50	–	–	100	–	–	100	–	–	–
NIGR	–	100	–	100	–	–	–	100	–	–	48	36	16	–
NIGR×TUIN	–	77	23	50	50	–	–	100	–	–	38	50	12	–
TUIN×TUBE	–	100	–	–	87	13	50	50	–	–	100	–	–	–
TUBE	–	100	–	–	100	–	100	–	–	3	85	12	–	–

Table 4. **Canonical discriminant statistics**

Discriminating between species and hybrids of the *Leptothorax tuberum* group using the variables head, femur, size of dark area on Ist gaster tergit, and demarcation of that area (see text). (For abbreviations of the species names see Tables 1–2)

% of variance	Func 1	Func 2	Func 3	Func 4
	81.49	17.22	1.26	0.03
Standardized coefficients				
Head	−0.0387	−0.2991	−0.2352	1.0189
Femur	0.8588	0.5440	−0.1359	−0.0462
Dark area size	0.2460	−0.2519	0.9978	−0.2241
Dark area demarcation	−0.4944	0.7906	0.2279	0.3231
Canonical functions evaluated at group centroids				
UNIF	−2.8489	3.8616	−1.0584	−1.3019
UNIF×TUIN	−3.0520	0.5669	−3.3622	0.2946
TUIN	−3.0026	−0.6595	−3.1737	0.0030
UNIF×NIGR	13.8206	−0.4346	−0.9505	0.3762
NIGR	15.0730	−6.6002	3.7634	2.2787
NIGR×TUIN	−2.5421	−5.4310	1.0150	1.6615
TUIN×TUBE	−3.4212	−5.2663	1.5303	0.5418
TUBE	−3.4757	−5.7807	1.7966	2.0254

Table 5. **The classification of the Leptothorax tuberum group species and hybrids as predicted from the discriminant analysis**

(For the abbreviations of the species names see Tables 1–2)

"Species"	Number of colonies classified as							
	UNIF	UNIF× TUIN	TUIN	UNIF× NIGR	NIGR	NIGR× TUIN	TUIN× TUBE	TUBE
UNIF	127	5	3	1				
UNIF×TUIN		5	2					
TUIN		6	11					
UNIF×NIGR				2				
NIGR					39			
NIGR×TUIN						5	3	3
TUIN×TUBE							2	
TUBE						5	5	23

Table 6. **Character scores mean and standard deviation, for workers of the Leptothorax tuberum group species and hybrids (see text)**

(For abbreviations of the species names see Tables 1–2; n= number of colonies studied)

"Species"	n	Head	Femur	Dark area on Ist gaster tergite	
				Size	Demarcation
		Scores 1–3	Scores 0–2	Scores 0–3	Scores 0–2
UNIF	136	1.3 (0.3)	0.0 (0.1)	1.0 (0.2)	1.9 (0.2)
UNIF×TUIN	7	1.7 (0.3)	0.0 (0.0)	0.4 (0.5)	1.1 (0.3)
TUIN	17	1.6 (0.4)	0.0 (0.0)	0.4 (0.4)	1.1 (0.3)
UNIF×NIGR	2	2.0 (0.0)	1.8 (0.2)	1.2 (0.3)	1.1 (0.1)
NIGR	39	2.9 (0.2)	2.0 (0.0)	2.7 (0.4)	0.0 (0.0)
NIGR×TUIN	11	2.3 (0.3)	0.1 (0.1)	1.8 (0.4)	0.1 (0.1)
TUIN×TUBE	2	2.0 (0.0)	0.0 (0.0)	1.8 (0.4)	0.1 (0.1)
TUBE	33	2.5 (0.4)	0.0 (0.1)	2.0 (0.5)	0.0 (0.0)

The 11 samples from loc. 7 are all heterozygous in MDH-1 (4 or more specimens/colony analyzed) clearly showing that they are hybrids, although they are morphologically typical *L. tuberum* (Table 2). From a morphological point of view *L. "tubero-interruptus"* should be one of the parents, contributing M to the MDH-1 heterozygote. This could also explain the rather high score for PGM (Table 2) and why the PGI S allele is found in some of these hybrid colonies (Table 3). The other parent has to be *L. nigriceps* being the only species with sufficiently high frequency of MDH-1 F (100%) to produce nothing but FM hybrids. Furthermore *L. nigriceps* was common in loc. 7. The queens in these hybrid colonies are typical *L. "tubero-interruptus"* in size (alitrunk length $\bar{x}$ = 1.17, SD = 0.05, n = 8; *L. nigriceps*, $\bar{x}$ = 1.28, SD = 0.04, n = 28) and colour (yellowish brown, not dark brown as in *L. nigriceps*).

The remaining two colonies in Table 2 contain only MDH-2 heterozygotes and could thus be hybrids between *L. tuberum* (the F allele) and most likely *L. "tubero-interruptus"*. The queens are small (alitrunk length x = 1.10 and 1.18; *L. tuberum* $\bar{x}$ = 1.23, SD = 0.04, n = 11) and almost as dark as in *L. tuberum* (dark brown).

The three isolated samples between *L. nigriceps* and *L. unifasciatus* in Fig. 2 are morphological intermediates between these species; rather dark head and slightly darkened femur (from *L. nigriceps*) and distinct gaster band (from *L. unifasciatus*). A hybrid origin of the two samples that are FM heterozygotes in MDH-1 is very likely, whereas the third sample (MS heterozygote) could be a back cross with *L. unifasciatus* (included in *L. unifasciatus* for the analysis below) (Fig. 4). Only in one of the samples (FM heterozygote) the queen was obtained, a typical *L. unifasciatus* queen.

So far we have assigned each sample to species or a specified hybrid using the available information from morphology and allozymes. The next step is to determine the morphological affinities between the species and the hybrids using a discriminant analysis.

Table 7. **No. of colonies of Leptothorax tuberum species and hybrids collected at 21 localities in Sweden and Germany (see Fig. 1)**

(For abbreviations of the species names see Tables 1–2)

Locality nr.	Number of colonies							
	UNIF	UNIF× TUIN	TUIN	UNIF× NIGR	NIGR	NIGR× TUIN	TUIN× TUBE	TUBE
1								11
2								16
3								3
4	18							
5	12	1			19			
6	3							
7	20	6	14		11	11		
8					2			
9	2							
10	4							
11	10		2					
12	1							
13	2							
14	5		1	1	3			
15	10							
16	4							
17	22			1	3			
18	22				1			
19	1							
20								1
21							2	2
Total	136	7	17	2	39	11	2	33

Tables 4 (group centroids) and 5 show five more or less distinct groups: 1. *L. unifasciatus* that overlaps the two following groups, 2. *L. unifasciatus* × *L. "tubero-interruptus* and *L. "tubero-interruptus"*, 3. *L. unifasciatus* × *L. nigriceps*, 4. *L. nigriceps*, and 5. *L. nigriceps* × *L. "tubero-interruptus"*, *L. tuberum* and *L. "tubero-interruptus"* × *L. tuberum*.

Although not a primary goal of this study we will briefly comment on how to discriminate between the species using the characters for workers presented here (Table 6). Only one species, *L. nigriceps*, is clearly separated from all the other including the hybrids. Apart from *L. nigriceps* a dark area on femora is found only in the hybrid with *L. unifasciatus* which has a dark gaster band (extensive dark area in *L. nigriceps* not formed as a band). A faint femoral melanism was also found in some hybrids with *L. "tubero-interruptus"*. *L. unifasciatus* is usually a characteristic species distinguished by its sharply delimited gaster band. Sometimes (in some populations) this band is more diffuse with a tendency to become interrupted in the middle thereby approaching the condition in *L. "tubero-interruptus"*. This similarity with *L. "tubero-interruptus"* might be due to introgression from the latter species since seven of the eight colonies of *L. unifasciatus* that were classified as either *L. unifasciatus* × *L. "tubero-interruptus"* or *L. "tubero-interruptus"* (Table 5) are from loc. 7, where all but one *L. unifasciatus* × *L. "tubero-interruptus"* were found (Table 7). *L. "tubero-interruptus"* differs from *L. tuberum* which has a darker head and a very diffuse dark area on gaster in contrast to the more or less marked, interrupted band in *L. "tubero-interruptus"*. *L. tuberum* cannot be separated from its supposed hybrid with *L. "tubero-interruptus"* nor from *L. nigriceps* × *L. "tubero-interruptus"*.

Discussion

From the morphological and electrophoretic analyses of our samples collected in South Sweden and Central and South Germany we conclude that there are four species in the *L. tuberum* group in Europe N of the Alps and W of approx. 15°. The distribution of these species is roughly as follows. *L. tuberum* is common in the north but rare in the southern parts. *L. unifasciatus* is the predominating species in the south (the most abundant species in 14 out of the 17 localities in Germany). *L. nigriceps* has a similar distribution but is less abundant. *L. "tubero-interruptus"* seems to be restricted to warm sites like the Rhine, Main and Tauber valleys (FISCHER 1987), and limestone areas (SEIFERT, pers. comm.; this study).

All four species occur in open to semi-open ground, *L. nigriceps* having a preference for more exposed sites than the other species (SEIFERT 1984, own obs.). The nests of *L. nigriceps* are found between stones and in rock crevices and this species is therefore confined to rocky/stony places. The other three species, especially *L. "tubero-interruptus"*, also inhabit dead wood (FISCHER 1987; SEIFERT, pers. comm.; own obs.).

At least three of the species, *L. unifasciatus*, *L. "tubero-interruptus"* and *L. nigriceps* interbreed. One of the hybrids, *L. unifasciatus* × *L. nigriceps*, can be identified on morphological grounds alone. So far, however, only three hybrid colonies have been reported (SEIFERT 1984; this study) suggesting that these species, frequently found together in the same habitat, rarely hybridize. Hybrid sexuals are produced (SEIFERT 1984), but it is not known if they are fertile. In any case, there is no evidence of gene flow from *L. unifasciatus* to *L. nigriceps*, since MDH-1 M (common in *L. unifasciatus*) was not found in *L. nigriceps*. Gene flow in the other direction might occur as indicated by the morphologically intermediate colony that was not an F_1 hybrid.

Although *L. nigriceps* × *L. "tubero-interruptus"* is distinct from its parents it is morphologically indistinguishable from *L. tuberum* (in the worker caste) and hence cannot be identified unless allozymes, karyotype or queen morphology is examined. The hybrid *L. "tubero-interruptus"* of FISCHER (1987) that he described as having slightly darker workers than pure *L. "tubero-interruptus"* is evidently *L. nigriceps* × *L. "tubero-interruptus"* as he

assumed. He reported this hybrid from two localities in South Germany. All 11 hybrid colonies in our study are from loc. 7, where both parent species occurred abundantly (Table 7). The approximately equal numbers of parent and hybrid colonies at this site suggest that *L. nigriceps* and *L. "tubero-interruptus"* easily hybridize when they co-occur. There are no indications, however, that hybrids backcross with any of the parents since *L. nigriceps* and *L. "tubero-interruptus"* were monomorphic for MDH-1 F and M, respectively.

In these cases of hybridization, where *L. nigriceps* is involved, there exists an asymmetry, as is the case in *Lasius niger* × *L. alienus* (PEARSON 1983), implying that *L. nigriceps*, offering the cheaper sex (males) is the least suffering party.

There is no doubt that *L. unifasciatus* and *L. "tubero-interruptus"* hybridize, but the precise nature of the hybrid colonies we found is not known, since there were no clearcut differences between the parent species (they mainly differed in PGM). Considering the difficulties in discriminating between *L. unifasciatus* and *L. "tubero-interruptus"* at the site, where we found six of the seven hybrid colonies, we believe that hybrid sexuals are fertil and backcross at least with *L. unifasciatus*. Thus, some of our hybrid colonies and the misclassified *L. unifasciatus* as well might have been the result of such backcrosses. It might be interesting to note, however, that laboratory crossings between these species produced but a few, small and short-lived workers (PLATEAUX 1978).

The only information we have about the inclination of *L. tuberum* to interbreed with other species is from the small sample from loc. 21 that was sent to us since the collector (B. SEIFERT) suspected two of the colonies to be *L. "tubero-interruptus"* × *L. tuberum* hybrids, as was later supported by the allozyme analysis. However, since we know almost nothing about allozyme variation in *tuberum* between Sweden and the Alps, it cannot be ruled out that the MDH-2 M allele is naturally occurring in *L. tuberum* and, hence, that these colonies are pure *L. tuberum*, although worker morphology in one of the colonies points to a hybrid origin.

Premating isolating mechanisms have apparently evolved between the two frequently co-occurring species *L. nigriceps* and *L. unifasciatus*, whereas *L. "tubero-interruptus"* easily interbreeds with these two species. The restricted distribution of *L. "tubero-interruptus"* might in fact be explained by the breeding competition from the other species. Since, as far as we understand, *L. "tubero-interruptus"* hardly can escape the presence of at least *L. unifasciatus*, the question is how *L. "tubero-interruptus"* can prevail to exist. We have no answer, but we recognize the fact that our study area comprises only a periferal part of the range of these species (AGOSTI and COLLINGWOOD 1987; BARONI-URBANI 1971; PLATEAUX 1978; own obs.) and that there might be ecological or other differences further south that prevent hybridization.

Acknowledgements

Our special thanks go to ALFRED BUSCHINGER who has helped us in many ways during our *Leptothorax* studies including the one presented here. We are also grateful to KARL FISCHER and BERNHARD SEIFERT who helped us collecting and to VERA ULVER who mounted the ants for the morphological analysis.

Zusammenfassung

Arthybride und Variabilität in der Leptothorax tuberum-Gruppe (Hymenoptera: Formicidae)

Anhand morphologischer und enzymelektrophoretischer Untersuchungen wurde festgestellt, daß in Nordwesteuropa vier verschiedene Arten der *Leptothorax tuberum*-Gruppe vorkommen: *L. tuberum* vorwiegend im Norden, aber *L. nigriceps*, *L. unifasciatus* und *L. "tubero-interruptus"* meist sympatrisch im Süden. Wir konnten auch nachweisen, daß Hybridisierungen vorkommen und Bastardvölker auftreten. *L. unifasciatus* und *L. nigriceps*, die häufig zusammen vorkommen, bastardieren selten, während *L. "tubero-interruptus"* offensichtlich leicht mit den anderen Arten kreuzt. Die Bastard-Arbeiterinnen nehmen in morphologischen Merkmalen eine Mittelstellung zwischen den Elternarten ein, doch ist der Bastard *L. nicriceps* × *L. "tubero-interruptus"* interes-

santerweise praktisch identisch mit *L. tuberum* bezüglich aller morphologischen Merkmale, die wir untersucht haben (Pigmentierung). Die Bedeutung der interspezifischen Kreuzungen wird diskutiert, und wir halten es für möglich, daß es einen Genfluß von sowohl *L. nigriceps* wie von *L. "tubero-interruptus"* zu *L. unifasciatus* gibt.

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Authors' address: PER DOUWES and BO STILLE, Department of Zoology, Helgonavägen 3, S-223 62 Lund, Sweden