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RESEARCH ARTICLE

Low genetic diversity in Polish populations of sibling ant species: *Lasius niger* (L.) and *Lasius platythorax* Seifert (Hymenoptera, Formicidae)

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Abstract We present preliminary data on mitochondrial DNA diversity within and among populations of the ants *Lasius niger* and *Lasius platythorax* in Poland. Phylogenetic analysis based on the mitochondrial DNA markers: *cytochrome c oxidase subunit I* (*cox1*) and *16S ribosomal RNA* (*16S rRNA*) confirms the species status of *L. niger* and *L. platythorax*. Intraspecific variability is low in both species, which might be a result of severe bottlenecks and rapid postglacial expansion into Central Europe.

Keywords *Lasius niger* · *Lasius platythorax* · Sibling species · mtDNA · Genetic diversity

Introduction

The sibling species *Lasius niger* and *Lasius platythorax* are widely distributed throughout the Palearctic ecozone. The latter species was described only as late as 1991 by Seifert. Despite their overall morphological similarity, the two species can be separated by discriminant analysis with various morphometric characters (Seifert, 2007). *Lasius niger* specializes in open habitats and nests mainly in soil, while *L. platythorax* is more common in woodland habitats and nests often in rotten wood. The two species are well separated with regard to ecological parameters (Seifert, 2007).

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There have been few molecular studies aiming to test phylogenetic relationships among Formicidae taxa including *Lasius* ants (Hasegawa, 1998; Hasegawa et al., 2002; Janda et al., 2004; Steiner et al., 2004; Maruyama et al., 2008). In these studies, only single sequences of individuals of *L. niger* species (mainly) and/or *L. platythorax* (rarely) were examined. Genetic data also were used to reconstruct the mating frequency of colony queens and paternity variation in the ant *Lasius niger* (Boomsma and Van der Have, 1998; Fjerdingstad et al., 2003). However, there is a lack of data on geographic distribution of genetic diversity in *L. platythorax* and these concerning *L. niger* are scarce. The phylogeography of ants has only recently received attention (Goropashnaya et al., 2004; Pusch et al., 2006).

Here, we present preliminary data on the mitochondrial DNA diversity within and among populations of the ants *L. niger* and *L. platythorax* in Poland. Using two mtDNA markers: *cytochrome c oxidase subunit I* (*cox1*) and *16S ribosomal RNA* (*16S rRNA*), we aimed at refining genetic relationships of *L. niger* and *L. platythorax*.

Materials and methods

The ant samples (workers) were collected from nests situated in north-east, Central and Southern Poland (Table 1) and individual specimens were classified on the basis of the description given by Seifert (1992) and Radchenko et al. (1999). Each locality was assumed to comprise a population. Individuals from three *L. niger* populations and two *L. platythorax* populations distant by at least 300 km were analyzed. Total DNA was extracted from the frozen specimens with Genomic Mini kit (A&A Biotechnology). The *cox1* and *16S rRNA* gene fragments were amplified using HCO2198/LCO1490 (Folmer et al., 1994), and

Table 1 Location of *L. niger* (*L.n*) and *L. platythorax* (*L.p*) species collection

Sample no.	Location		Environment/humidity	Nest type
L.n 067	Górki (Kampinoski National Park)	52°19′N, 20°31′E	Open area; meadow/dry	In soil
L.n 068	Granica (Kampinoski National Park)	52°17′N, 20°27′E	Open area; dry	In soil
L.n 074	Polichty (Ciężkowicko-Rożnowski Landscape Park)	49°48′N, 20°52′E	Open area; big clearing/dry	In soil
L.p 075	Lubasz	50°17′N, 21°04′E	Forest area; moderately damp	In rotten trunk
L.n 077	Krzywe	54°05′N, 23°00′E	Open area; lawn/dry	In soil
L.n 101	Krzywe	54°04′N, 23°00′E	Open area; field/dry	In soil
L.n 106	Krzywe Zdroje	54°05′N, 22°59′E	Open area; meadow/dry	In soil
L.n 107	Krzywe Zdroje	54°05′N, 22°58′E	Open area; meadow/dry	In soil
L.p 102	Sec.275 (Wigry National Park)	54°00′N, 23°09′E	Forest area; moderately damp	In rotten trunk covered with moss
L.p 103	Sec.275 (Wigry National Park)	54°00′N, 23°09′E	Forest area; moderately damp	In rotten trunk covered with moss
L.p 104	Sec.275 (Wigry National Park)	54°00′N, 23°09′E	Forest area; moderately damp	In rotten trunk covered with moss
L.p 105	Sec.276 (Wigry National Park)	54°00′N, 23°09′E	Forest area; moderately damp	In broken tree's trunk

16Sbr/16Sar-Dr (Palumbi and Benzie, 1991) primer pairs, respectively. The annealing conditions were set to 49°C for 1 min (*cox1*) and 52°C for 1 min (*16S rRNA*). PCR products were sequenced directly from both directions, using the same primers as at the amplification stage.

All newly obtained sequences were deposited in GenBank (accession nos: GQ503244–GQ503250). Sequence alignment was achieved with BioEdit (Hall, 1999). For the phylogenetic analyses, 680 bp of *cox1* and 401 bp of *16S rRNA* fragments were used. To infer phylogenetic relationships, neighbour-joining algorithm (NJ), maximum parsimony analysis (MP) with MEGA 4.0 (Tamura et al., 2007) and maximum likelihood method (ML) with TREE-PUZZLE 5.1 (Schmidt et al., 2002) were performed. Tamura-Nei distance (D_{TrN}; Tamura and Nei, 1993) was used for the NJ trees. Statistical confidence in nodes was calculated by bootstrap resampling with 1,000 replicates (Felsenstein, 1985) or quartet puzzling steps for TREE-PUZZLE.

Based on programs ModelTest 3.1 (Posada and Crandall, 1998) and PAUP (Swofford, 2002) the substitution model Tamura-Nei (TrN), with equal rates for all sites in both dataset (*cox1* and *16S rRNA*), was used. Data from both regions were analyzed separately and then combined and analysed together (Fig. 1). Based on the phylogenetic studies for ants (Steiner et al., 2004; Maruyama et al., 2008) *Lasius emarginatus* sequences were used as an outgroup (accession nos: AB371057, AY225868).

The analyses were also complemented by adding previously published sequences of the gene regions targeted, retrieved from GenBank: *cox1*; accession nos.: AB007981 (Hasegawa, 1998; Hasegawa et al., 2002), AY225866–AY225867 (Steiner et al., 2004), AB371019-AB371020

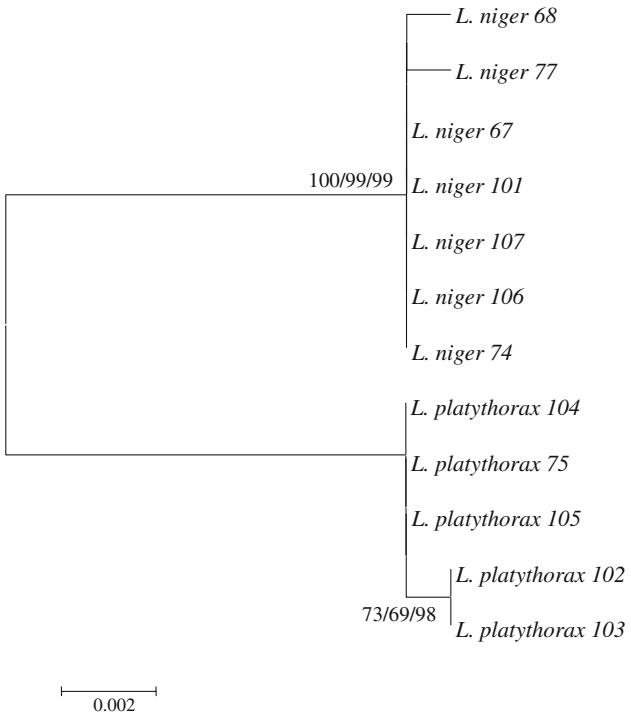

Fig. 1 Neighbour-joining (NJ) phylogenetic tree of combined data of region *cox1* + *16S rRNA* calculated with TrN algorithm. Number after species name indicates localities for particular haplotype/population. Bootstrap values >65 were given at nodes for NJ and MP (first and second values) and quartet puzzling steps for ML phylogenetics methods (third value)

(Maruyama et al., 2008) and *16S rRNA*; accession nos.: AB371065-AB371066 (Maruyama et al., 2008). However, for the *cox I* sequence alignments the 379 bp overlap with

sequences reported by Steiner et al. (2004) and the 283 bp overlap with sequences reported by Maruyama et al. (2008) were possible. For *16S rRNA* data, the overlap with sequences deposited in GenBank was complete (401 bp).

Results

In case of *cox1* gene region, analyses were based on 14 variable sites (12 informative sites) for only third codon position. All mutational changes were base pair substitutions, with about 71.4% of *Ti*s. No mutation led to a change at the amino acid level. Within *L. niger* samples, two different haplotypes were detected with a single base change separating *L. niger* 77 colony (accession no: GQ503248) from the rest of colonies (Fig. 1). A similar situation was observed also in the case of *L. platythorax*; *L. platythorax* 102 and 103 colonies (accession no: GQ5032549) show a single nucleotide substitution (Fig. 1). Table 2 reports the pairwise distance D_{TrN} values. The sequences were shortened (different pairs of primers were used in the *L. emarginatus* studies); however, the distance values remained unchanged.

In case of *16S rRNA* region, analyses were based on eight variable sites (seven informative sites). All mutational changes were base pair substitutions, with about 75% of *Ti*s. Within *L. niger* colonies, two different haplotypes were found with a single base change separating *L. niger 68* colony (accession no: GQ503245) from the rest of *L. niger* colonies (Fig. 1). In *L. platythorax* samples, only one representative haplotype was observed. Table 3 reports the pairwise distance D_{TrN} values.

As both mtDNA regions analysed gave the same result, the data were pooled and one combined phylogenetic tree was constructed (Fig. 1). The congruence of the tree topologies generated by different phylogenetic methods suggests that this is a reliable estimate of true relationships.

Table 2 Pairwise distance TrN calculation (D_{TrN}) for *cox1* partial region (379 bp)

	L. n I	*L. n II*	*L. p I*	*L. p II*
L. n I				
L. n II	0.003			
L. p I	0.019	0.022		
L. p II	0.022	0.025	0.003	
L. e	0.024	0.027	0.024	0.027

L.n, *L. niger*; *L.p*, *L. platythorax*; *L.e.*, *L. emarginatus* (AY225868)

Haplotype: *L. n I (L.n67, L.n68, L.n74, L.n106, L.n107, L.n101)*; *L. n II (L.n77); L. p I (L.p75, L.p104, L.p105); L.p II (L.p102, L.p103)*

Number after species name indicates localities for particular sample

Table 3 Pairwise distance TrN calculation (D_{TrN}) for *16S rRNA* partial region (401 bp)

	L. n I	*L. n II*	*L. p*
L. n I			
L. n II	0.003		
L. p	0.016	0.018	
L. e	0.021	0.023	0.015

L.n, *L. niger; L.p*, *L. platythorax; L.e.*, *L. emarginatus* (AB371057)

Haplotype: *L. n I (L.n67, L.n74, L.n77, L.n106, L.n107, L.n101)*; *L. n II (L.n68); L. p (L.p75, L.p102, L.p103, L.p104, L.p105)*

Number after species name indicates localities for particular sample

Discussion

The sequences of the *cox I* and *16S rRNA* regions for all the specimens studied were found to fall into two monophyletic groups. The range of sequence divergences (Tables 2, 3) between *L. niger* and *L. platythorax* suggests that both species have been well separated though the time of interspecific diversification appears to be a rather recent. If we applied (after Pusch et al., 2006) the honey bee rate of mtDNA divergence, 2% per million years (Arias and Sheppard, 1996), these species would have diverged about 1 Mya.

The recent morphological and genetic data revealed the surprising commonness of hybridization in ants (Feldhaar et al., 2008; Seifert, 2009), also among members of the genus *Lasius* (Pearson, 1983; Seifert, 1999; Umphrey, 2006). It seems plausible that in case of recently diverged species, as *L. niger* and *L. platythorax,* the reproductive barriers cannot completely prevent the possibility of interspecific hybridization. There are reports that both species *L. niger* and *L. platythorax* may hybridize with other ant species, i.e., *L. niger* with *L. alienus* (Pearson, 1983) or *L. psammophilus* (Seifert, 1999), and *L. platythorax* with *L. emarginatus* (Seifert, 1999). There are even some observations of morphologically intermediate forms between *L. niger* and *L. platythorax* inhabiting the moss-covered rock cracks in Finland (W. Czechowski, pers. comm.). However, there is no scientific evidence of hybridization between both *Lasius* species.

With respect to geography, the hybridization may occur sporadically between broadly sympatric species or be limited to particular contact zone (Avise, 2004). In case of *L. niger* and *L. platythorax*, both species occur in close geographic proximity and also our observations confirm their sympatry; the colonies of both species can be situated even less than 50 m apart. On the other side, ant species which maintained interspecific contact during their natural history have built up more effective mechanisms of prezygotic isolation than species that rarely or never experienced such contact (Seifert, 1999). In consequence, the

observed frequency of hybridization is lower in the first group of species (Seifert, 1999). The chances for interspecific hybridization may also depend on the time limitation for mating activities. *L. niger* and *L. platythorax* do not differ in the timing of their sexual activity, resulting in the possibility of co-occurrence of sexuals of both species. Moreover, in the north-eastern part of Poland we observed that even the daily hours of their nuptial flights may overlap. Both sibling species are monogynous and monoandrous, what possibly leaves less opportunities for interspecific hybridization. However, in southern Germany, *Temnothorax* hybrid workers were frequently found among workers of pure-species *T. nylanderi* or *T. crassispinus*, despite obligate monogyny and monandry in both sibling species (Pusch et al., 2006). Although there are no detailed studies, the hybridization between *L. niger* and *L. platythorax* seems also probable and only detailed research based on more samples and a wider study area could shed more light on this topic.

Along with marked interspecies divergence between *L. niger* and *L. platythora*x, low intraspecific variation across the examined range was observed. In our analyses of combined data (*cox I* + *16S rRNA*), 57% of *L. niger* haplotypes were identical and 60% of *L. platythorax*. In comparison, two other sibling ant species widespread in Europe: *T. crassispinus* and *T. nylanderi*, showed similar genetic divergence values in *cox I* haplotypes (2.4%) but no more than 40% haplotypes of both species were identical (Pusch et al., 2006). Moreover, when we complemented our analyses by adding the *cox I* and *16S rRNA* sequences obtained from specimens of *L. niger* and *L. platythorax* from Austria (Steiner et al., 2004; Maruyama et al., 2008), we found that despite the long geographical distance, the most common Polish haplotypes of both species appeared to be identical to previously mentioned haplotypes deposited in GenBank.

In one case, the *cox I* haplotype of *L. niger* (Hasegawa, 1998; Hasegawa et al., 2002), we noted the 3% sequence divergence (for 470 bp sequence alignment) between the haplotype mentioned and the cluster of *L. niger* and *L. platythorax* Polish haplotypes. If we assumed that this haplotype represents correctly classified species, such a level of *L. niger* intraspecific variation could be the result of local differentiation processes, often observed for geographically widely distributed species (e.g., Ross and Shoemaker, 2005; Burns et al., 2008). However, the place of origin of the *L. niger* sample concerned appears to be unclear. The sample comes either from Spain (Hasegawa (1998) or from Germany (Hasegawa et al., 2002). This uncertainty makes inferring hardly possible.

Generally, geographically restricted species are characterized by lower genetic diversity, than more widespread species. However, in the present study we observed widely distributed common haplotype and few unique haplotypes that resemble typical star-like phylogeny. This phylogeny pattern and low diversity in both species strongly suggest a recent bottleneck event followed by, most probably postglacial, population expansion (e.g. Goropashnaya et al., 2004). Our results showing low intraspecific variation are also concordant with the preliminary allozyme analyses of four gene loci (*Pgm, Idh, Mdh, Pgi*) from Polish samples of *L. niger* and *L. platythorax* (A. Wysocka, unpubl. data). However, there is not enough data to draw inferences about the level of gene flow and introgression between the populations studied.

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