

A NEW POLYGYNOUS *LASIUS* SPECIES  
(HYMENOPTERA : FORMICIDAE)  
FROM CENTRAL EUROPE

II. ALLOZYMATIC CONFIRMATION OF SPECIES STATUS  
AND SOCIAL STRUCTURE

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SUMMARY

The ant species *Lasius* (*Lasius*) *neglectus* spec. nov. (VAN LOON *et al.*, 1990) was sampled from its type locality in Budapest, Hungary for allozyme analysis at four marker loci. *L. neglectus* appeared to be monomorphic but different from *L. alienus* at three loci. The fourth locus, *Me*, was found to be polymorphic in *L. neglectus* and was used to make a preliminary assessment of population viscosity, intranest relatedness, and mating structure. Allele frequencies turned out to be homogeneous across mother-queens, males and workers. Between sample sites, the *Me* allele frequencies of workers were more heterogeneous and some population viscosity could be detected. The mean intranest relatedness of *L. neglectus* was found to be very low, both between workers and between workers and males, but no indications were found that queens are frequently inseminated more than once. The analysis of worker genotypes further showed a significant effect of inbreeding. The results are generally consistent with the observations about polygyny and intranidal mating reported by VAN LOON *et al.* (1990). Some striking similarities between *L. neglectus* and the Japanese *L. sakagami* — the only other polygynous *Lasius* s.s. known to science — are discussed.

RESUME

Une nouvelle espèce polygyne de *Lasius* (Hymenoptera : Formicidae) d'Europe centrale  
II. Confirmation allozymatique du statut spécifique et structure sociale

Nous avons récolté des échantillons de *Lasius* (*Lasius*) *neglectus* spec. nov. dans la localité type à Budapest, Hongrie, pour effectuer une analyse des allozymes. Nous avons étudié quatre loci. Trois loci de *L. neglectus* sont monomorphes et différents de *L. alienus*.

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L'autre locus, l'enzyme malique (*Me*), est polymorphe chez *L. neglectus*. Ce locus a été utilisé afin d'étudier la viscosité de population, le coefficient de parenté entre ouvrières du même nid et les modalités d'accouplement. Les fréquences d'allèles sont homogènes entre les reines, les mâles et les ouvrières. Les fréquences de génotypes chez les ouvrières sont plus hétérogènes entre les nids et on peut détecter une certaine viscosité de la population. Dans les nids de *L. neglectus*, le coefficient de parenté entre ouvrières et entre ouvrières et mâles est très faible. Les reines semblent n'être inséminées que par un seul mâle. L'analyse de génotypes des ouvrières indique aussi l'existence d'une certaine consanguinité. En général, les résultats correspondent avec les observations sur le terrain (polygynie et accouplement dans le nid) (voir VAN LOON *et al.*, 1990). Nous comparons dans la discussion la structure sociale de *L. neglectus* avec celle de l'unique autre espèce de *Lasius* s.s. polygyne connue (*L. sakagamii*).

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## INTRODUCTION

Species of the genus *Lasius* s.s. (or subgenus *Lasius*) are among the most common ants in the Holarctic region (WILSON, 1955). The hitherto known European representatives of this subgenus, *L. niger*, *L. alienus*, *L. brunneus* and *L. emarginatus* tend to be both monogynous and monodomous (e.g. COLLINGWOOD, 1979), but polydomy has been reported for the monogynous North American species *L. neoniger* by TRANIELLO and LEVINGS (1986). The first polygynous species of *Lasius* s.s. was only rather recently described from Japan (YAMAUCHI and HAYASHIDA, 1970). Workers of this ant, *L. sakagamii*, were shown to be morphologically most related to *L. niger*, but to be much more hairy overall. In a later paper, YAMAUCHI *et al.* (1981) reported that this species is both extremely polygynous and polydomous and that it was very different from *L. niger* in behaviour and ecology. Apart from its social organization, the most striking characters of *L. sakagamii* are its apparent ability to monopolize local floodplain habitats and the absence of distinct colony boundaries.

In the preceding paper by VAN LOON *et al.* (1990) a new polygynic species, *Lasius neglectus*, was described from Budapest, Hungary. The workers of this ant are very similar to *L. alienus*, in external morphology, but their foraging ecology seems to be more like that of *L. brunneus*. Also, *L. neglectus* was shown by VAN LOON *et al.* to monopolize suburban habitats and to have unclear colony boundaries. Unlike *L. sakagamii*, however, the natural distribution of *L. neglectus* is yet unknown. There are some indications that this ant was introduced into the Budapest area some fifteen to twenty years ago. Although the morphological differences between the sexuals of *L. alienus* and *L. neglectus* — as described in the preceding paper — are distinct enough to justify its status as a separate species, it remained unclear whether *L. neglectus* is indeed closely related to *L. alienus* and whether the social structure of the Budapest population is as similar to that of *L. sakagamii* as the observational data of VAN LOON *et al.* suggest.

This paper therefore reports a first and preliminary analysis of allozyme variation in *L. neglectus* and a comparison of the patterns in this species with those in sympatric populations of four other species of the subgenus *Lasius* and a fifth — probably also new — species that has not yet been properly named. The data of one polymorphic enzyme-locus are used to assess some details on intranest relatedness, population viscosity (HAMILTON, 1964) and mating structure.

### METHODS

Individuals of *Lasius neglectus* were sampled from nests at the type locality, which is the garden around the Company for the Development of Fruit and Ornamental Production in the Budatétény quarter of the city of Budapest, Hungary (see VAN LOON *et al.*, 1990). Samples of workers and, if present, sexual ants were collected in July 1987, July 1988 and July 1989. The different samples (minimal distance ca 10 m) represent separate nests in an assemblage which is likely to be a mosaic of polydomous colonies (see also VAN LOON *et al.*, 1990). As shown in figure 1, the different sets of samples cover the entire distribution area of this ant and are thus considered to be representative. In 1989, twenty samples were deliberately taken in a gradient — starting at the type-

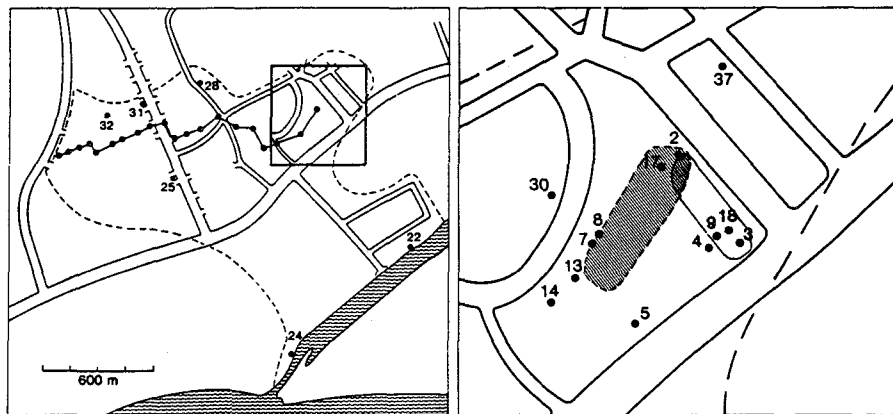


Fig. 1. — Distribution in 1988 of *Lasius neglectus* in Budapest (see also fig. 1 in VAN LOON *et al.*, 1990), where numbers refer to the nests sampled in 1988. The 1987 and 1989 sample sites were only approximately recorded and are indicated by the hatched areas (dashed and solid margins, respectively). The 1989 sampling area is approximately the same as the type locality in VAN LOON *et al.* (1990). The 20 additional samples taken in 1989 in a gradient from the type locality to the periphery are also indicated.

Fig. 1. — Distribution de *Lasius neglectus*, en 1988 à Budapest (voir aussi la fig. 1 de l'article VAN LOON *et al.*, 1990; les numéros font référence aux nids échantillonnés en 1988. Les nids échantillonnés en 1987 et 1989 ont été localisés approximativement et sont indiqués par une zone hachurée (délimitée respectivement par un trait discontinu et continu). Les échantillons de 1989 ont été prélevés à peu près dans la localité type telle qu'elle est indiquée par VAN LOON *et al.*, 1990. Les vingt échantillons supplémentaires prélevés en 1989 selon un gradient à partir de la localité type vers la périphérie, sont également indiqués.

locality and extending towards the margin of the current distribution — to assess whether there was any systematic pattern in allele-frequencies along this inferred colonization gradient. The samples of the five sympatric *Lasius* s.s. species were collected in July 1989 at various localities in and around Budapest.

Nest samples were either stored alive at 5° C or frozen at -60° C until used for starch-gel electrophoresis. For that purpose whole males and the head plus mesosoma of workers and gynes were ground in 25, 15 and 50 µl distilled water, respectively. The homogenate was then absorbed onto 4 × 11 mm pieces of Whatman 3MM filter paper before being inserted in the (horizontal) gels. Four enzyme systems, Malic enzyme (*Me*), Esterase (*Est-2*), and Malate dehydrogenase (*Mdh*) and Phosphoglucose mutase (*Pgm*) were analysed, using buffer systems and staining techniques of MENKEN (1982).

## RESULTS

The comparative analysis of allozyme variation in the European species of the subgenus *Lasius* (table I) showed that *L. neglectus* is very distinct from all its congeners. It has the unique 101- and 107-allele for *Me* and *Pgm*, respectively, and can also be readily identified from the fixed 95-allele for *Est-2*. In fact, both *L. neglectus* and the yet unnamed "species-1" tend to be more different from the other species than the other species differ among themselves. However, also the other *Lasius* species have their own unique combination of allozymes: *L. alienus* is characterized by fast alleles for both *Est-2* and *Mdh*, *L. niger* by its polymorphism for *Me* with a relatively high proportion of slow alleles, *L. brunneus* has a unique fast allele for *Pgm* and *L. emarginatus* combines a limited polymorphism for *Est-2* with a virtually monomorphic *Me*-locus.

The overall distribution of *L. neglectus* genotypes for the polymorphic *Me*-locus is given in table II. Significant deviations of nest-samples from the overall genotype-frequencies observed in the respective sets of samples were present but proved to be relatively rare, especially if one bears in mind that a certain amount of "significant differences" tend to appear by chance in multiple comparisons like this. Still, the pooled heterogeneities per set of samples were significant for the worker-data:  $G = 66.95$  (df = 22;  $P < 0.001$ ) in 1987,  $G = 105.78$  (df = 38;  $P < 0.001$ ) in 1988 and  $G = 98.33$  (df = 64;  $P < 0.01$ ) in 1989. In queens and males, however, the nest deviations in genotype distribution were even less pronounced than in workers, and the overall heterogeneity was not significant (males:  $G = 18.76$ , df = 14,  $P > 0.1$ ; queens:  $G = 17.38$ , df = 20,  $P > 0.5$ ).

The deviating frequencies in part of the worker-samples suggests that there could be some viscosity in this population due to e.g. limited dispersal. To investigate this in somewhat more detail we also analysed the 1988 pattern of genotypic variation among nests by regressing the genetic identity  $I_{ME}$  of workers of each pair of sites against the metric distance between them (see PAMILO and ROSENGREN, 1984). This regression was found to be non-significant if all possible pairs of sites were included (slope = -0.013;  $P > 0.5$ ), but turned out to be significant when the analysis was restricted

Table I. — Allele frequencies at four marker loci, Malic enzyme (*Me*), Esterase (*Est-2*), anodal Malate dehydrogenase (*Mdh*) and Phosphoglucumutase (*Pgm*) in workers of the new species *Lasius neglectus* and its five congeneric species of the subgenus *Lasius*. Sample size (*n*) refers to worker-number with number of colonies in brackets. All allozyme-numbers refer to the relative distance to the most common 100-allele in *L. niger*.

Tableau I. — Fréquences allélique pour l'Enzyme Malique (*Me*), l'Esterase (*Est-2*), la Malate dehydrogenase anodal (*Mdh*) et la Phosphoglucumutase (*Pgm*) chez les ouvrières de la nouvelle espèce *Lasius neglectus* et de cinq espèces congénériques de *Lasius*. *n* représente la taille de l'échantillon (nombre d'ouvrières traitées et nombre de colonies entre parenthèses). Les chiffres relatifs aux allozymes représentant la distance relative à l'allèle 100 le plus commun chez *L. niger*.

	allele	<i>L. neglectus</i>	<i>L. alienus</i>	<i>L. niger</i>	<i>L. emarginatus</i>	<i>L. brunneus</i>	<i>L. 1</i> *
<i>Me</i>	96	—	0.01	0.17	0.01	—	1.00
	100	0.84	0.99	0.83	0.99	1.00	—
	101	0.16	—	—	—	—	—
	<i>n</i>	439 (30)	66 (7)	56 (9)	118 (10)	139 (11)	166 (13)
<i>Est-2</i>	85	—	—	—	—	—	0.28
	90	—	—	—	—	—	0.28
	95	1.00	—	—	—	—	0.37
	100	—	0.07	0.66	0.85	—	0.06
	105	—	—	0.25	0.15	—	—
	110	—	0.93	0.10	—	—	—
	<i>n</i>	248 (23)	22 (3)	151 (14)	132 (12)	89 (8) **	203 (17)
<i>Mdh</i>	96	—	0.09	—	0.26	0.17	0.23
	100	1.00	0.87	1.00	0.74	0.83	0.77
	104	—	0.03	—	—	—	—
	<i>n</i>	263 (29)	74 (8)	66 (7)	146 (12)	141 (11)	160 (13)
<i>Pgm</i>	100	—	1.00	1.00	1.00	—	1.00
	107	1.00	—	—	—	—	—
	110	—	—	—	—	1.00	—
	<i>n</i>	59 (6)	1 (1)	11.2	9 (3)	31.3	5 (2)

\* Another *Lasius* ss. species of unknown status (species 1 in Agosti and Collingwood, 1987).

\*\* *Est-2* could never be stained for *L. brunneus*. The slower system *Est-1*, however, was active but monomorphic in all sampled colonies of *L. brunneus*. The only other species which had *Est-1* was *L. emarginatus*. Here the system was polymorphic, showing three alleles at frequencies of 0.66, 0.27 and 0.07 (from slow to fast). The rare fast allele was the same as the one in *L. brunneus*.

to distances below 500 m, excluding most samples from outside the garden around the Company for the Development of Fruit and Ornamental Production (slope = -0.599;  $P < 0.001$  one-tailed).

We also tested for patterns in the allele-frequencies along the 1989 gradient of samples (from center to periphery). It appeared that the frequency of the *Me*-100 allele increased significantly with the distance of the samples from the type locality ( $P < 0.001$ ), such that only 100/100 nests were found at the end of the gradient. This confirmed our earlier impression from the 1988 samples (table II), where also a number of the

Table II. — Genotype distribution at the Malic enzyme (*Me*) locus for workers, males and queens of *Lasius neglectus* in samples taken in July 1987, July 1988, and July 1989 at the sites indicated in fig. 1. Significant deviations of samples from the overall genotype frequency are indicated (G-tests; \*:  $P < 0.05$ ; \*\*\*:  $P < 0.001$ ).

Tableau II. — Distribution des génotypes pour l'enzyme malique (*Me*) chez les ouvrières, mâles et reines de *Lasius neglectus* dans des échantillons récoltés en juillet 1987, juillet 1988 et juillet 1989 aux localités indiquées dans la fig. 1. Les déviations significatives d'échantillons par rapport à la fréquence génotypique générale sont indiquées (G-tests; \*:  $P < 0,05$ ; \*\*\*:  $P < 0,001$ ).

nest	queens			workers			males	
	100/100	100/101	101/101	100/100	100/101	101/101	100	101
87-A	—	—	—	4	10	0 ***	—	—
87-B	—	—	—	13	2	0	—	—
87-C	—	—	—	2	0	0	—	—
87-D	19	3	0	28	0	0 ***	6	0
87-E	—	—	—	16	2	0	—	—
87-F	1	0	0	15	1	0	—	—
87-G	—	—	—	34	6	6	—	—
87-H	—	—	—	5	2	2	—	—
87-I	—	—	—	7	5	2	—	—
87-J	—	—	—	9	4	3	—	—
87-K	—	—	—	10	9	1	—	—
88-2	—	—	—	7	3	0	21	3
88-3	—	—	—	10	2	2	21	3
88-4	—	—	—	15	2	0	8	2
88-5	—	—	—	18	4	0	16	0 *
88-7	—	—	—	14	2	0	1	1
88-8	—	—	—	19	8	0 *	16	4
88-9	—	—	—	7	0	1	23	3
88-13	—	—	—	5	0	0	18	0 *
88-14	—	—	—	10	0	0	13	3
88-17	—	—	—	10	3	2	21	8
88-18	—	—	—	16	2	1	21	4
88-22	—	—	—	13	1	3	—	—
88-24	—	—	—	12	0	0 *	—	—
88-25	—	—	—	7	0	0	—	—
88-28	—	—	—	7	0	7 ***	—	—
88-30	—	—	—	9	2	1	—	—
88-31	—	—	—	1	1	0	—	—
88-32	—	—	—	4	0	0	—	—
88-37	—	—	—	0	10	0 ***	—	—
89-A	6	1	0	3	0	0	—	—
89-B	8	0	0	9	0	0	—	—
89-C	10	4	0	3	0	0	—	—
89-D	—	—	—	7	1	1	—	—
89-E	8	0	0	2	0	0	—	—
89-F	4	1	0	6	0	0	—	—
89-G	—	—	—	10	2	0	—	—
89-H	9	1	0	3	0	0	13	3
89-I	—	—	—	1	3	0 *	—	—
89-J	—	—	—	5	0	0	—	—
89-K	6	1	2 *	10	3	3 *	5	1
89-L	10	2	0	6	4	0	—	—

89 - g1	—	—	—	9	4	0	—	—
89 - g2	—	—	—	2	2	0	—	—
89 - g3	—	—	—	10	3	3 *	—	—
89 - g4	—	—	—	4	2	0	—	—
89 - g5	—	—	—	2	3	0	—	—
89 - g6	—	—	—	7	0	0	—	—
89 - g7	—	—	—	11	6	0	—	—
89 - g8	—	—	—	7	2	0	—	—
89 - g9	—	—	—	12	0	0	—	—
89 - g10	—	—	—	7	0	0	—	—
89 - g11	—	—	—	8	2	0	—	—
89 - g12	—	—	—	4	1	1	—	—
89 - g13	—	—	—	6	0	0	—	—
89 - g14	—	—	—	12	0	0	—	—
89 - g15	—	—	—	7	1	0	—	—
89 - g16	—	—	—	10	0	0	—	—
89 - g17	—	—	—	10	0	0	—	—
89 - g18	—	—	—	9	0	0	—	—
89 - g19	—	—	—	12	0	0	—	—
89 - g20	—	—	—	10	0	0	—	—

outermost nests contained only 100/100 individuals. Regression of genetic identity  $I_{ME}$  on ranked distance along the gradient was marginally significant (slope = -0.0017;  $P < 0.1$  one-tailed test). These results indicate that population viscosity does occur in this *L. neglectus* population, but that its detection apparently depends on the scale of observation and the density of sampling.

Of the queens collected in nest D in 1987 (see *table II*), eleven dealated individuals survived rearing in isolation to produce a worker brood on their own. After that, allozymatic mother-offspring analysis was performed to check the constancy of allele frequencies across generations and to see whether multiple matings could be detected. The results (*table III*) suggest that the queens were singly inseminated, since all offspring of homozygous mothers have the same genotype and the offspring of heterozygous queens consist of ca 50% homozygotes and 50% heterozygotes. Furthermore, it was found that the allele frequencies were the same for the inferred single fathers, mothers and offspring involved in the sample of *table III*, and that these frequencies rather accurately reflected those found in the samples of the mature nests (*table IV*).

Finally, the regression coefficient of relatedness between workers ( $b'_{tt}$ ) and between workers and males ( $b'_{mf}$ ) was estimated following the calculation procedure of PAMILO and CROZIER (1982). Because of the substantial unplanned variation in the number of individuals collected for electrophoresis (see *table II*) we only used estimates weighted by sample size. Standard errors of  $b'$  were estimated with a jackknife method according to CROZIER *et al.* (1984). Relatedness between workers ( $b'_{tt}$ ) was estimated to be  $0.138 \pm 0.052$  for the pooled samples of 1987 and 1988 ( $n = 30$ ) and  $0.140 \pm 0.037$  for the 1989 samples ( $n = 32$ ). Both estimates are significantly different from zero

Table III. — Genotype distribution at the Malic enzyme (*Me*) locus for eleven dealate queens of *Lasius neglectus* and their worker offspring. All queens were from nest 87-D (see *table II*). The inferred paternal genotypes are also given, under the assumption that all queens were singly inseminated.

Tableau III. — Distribution des génotypes de l'enzyme malique (*Me*) pour onze reines désailées de *Lasius neglectus* et leurs ouvrières filles. Toutes les reines sont du nid 87-D (voir *tableau II*). Les génotypes paternels déduits sont donnés aussi en supposant que toutes les reines sont inséminées une seule fois.

Queen genotype	Worker-offspring genotypes 100/100	100/101	Inferred paternal genotype
100/100	10	0	100
100/100	8	0	100
100/100	6	0	100
100/100	6	0	100
100/100	5	0	100
100/100	3	0	100
100/100	0	6	101
100/100	0	3	101
100/101	4	3	100
100/101	2	5	100
100/101	3	3	100

Table IV. — Frequencies of the most common (100) allele of Malic enzyme (*Me*) according to estimates in the various samples of *table II* and *III*.

Tableau IV. — Fréquences de l'allèle le plus commun (100) à l'enzyme malique (*Me*) selon des estimations dans les échantillons de *tableau II* et *III*.

Queens collected in 1987 and 1989 ( <i>table II</i> )	0.911
Eleven reared queens ( <i>table III</i> )	0.864
Workers collected in 1987 and 1988 ( <i>table II</i> )	0.837
Workers collected in 1989 ( <i>table II</i> )	0.899
Workers reared experimentally ( <i>table III</i> )	0.851
Males collected in 1987, 1988 and 1989 ( <i>table II</i> )	0.853
Inferred fathers of reared workers ( <i>table III</i> )	0.818

in one-tailed tests ( $P < 0.01$  and  $< 0.001$ , respectively). However, the *L. neglectus* workers were also inbred to some extent (weighted  $F = 0.323 \pm 0.111$  for the 1987/1988 samples and  $0.211 \pm 0.126$  for the 1989 samples. This implies that the expected value of  $b'_{ff}$  in the absence of inbreeding would be even lower than 0.14 (PAMILO, 1985). The relatedness between workers and males ( $b'_{mf}$ ) was found to be about zero ( $0.014 \pm 0.020$ ;  $n = 14$ ).

The mean genetic relatedness was also estimated for workers from pairs of neighbouring nests. For the 1988 samples, this was done separately for five pairs of very close nests (distance  $< 35$  m) and 14 pairs of moderately distant nests (distance  $< 500$  m), but in both cases  $b'_{ff}$  was found to be not significantly different from zero ( $0.06 \pm 0.10$  and  $-0.01 \pm 0.05$ , respectively).



The neighbouring nests in the 1989 gradient produced a similar result ( $b'_{it} = 0.09 \pm 0.06$ ).

## DISCUSSION

The results of *table I* show that *Lasius neglectus* can be discriminated from sympatric populations of *L. alienus* and other congenics by at least one diagnostic allozyme. This is sufficient to confirm its status as a separate species and the same argument can be made for the yet unnamed "species 1". It also indicates that *L. neglectus* is not very closely related to its morphologically most similar congener *L. alienus*, and that the evolution of the *neglectus* polygyny syndrome is not a very recent event as e.g. in the North American populations of the fire ant *Solenopsis invicta* (Ross and FLETCHER, 1985).

Offspring genotypes are maximally heterogeneous when they all have the same mother and father within families (or colonies or nests), but different parents across families. The pattern given in *table III* resembles this theoretically expected heterogeneity for haplodiploid full sib families very closely, i.e. offspring are either all homozygous or half homozygous and half heterozygous. Examples of similar offspring genotype-distributions are given by VAN DER HAVE *et al.* (1988) for both incipient and mature colonies of *L. niger*. In cases like that, *G-tests* on nest-samples of similar size as the ones in *table II* generally yield significant deviations from the overall genotype frequency in about 90% of the nests ( $P < 0.001$  in 70% of the nests). Obviously, the heterogeneity of genotypes across mature nests of *L. neglectus* (*table II*) is much less pronounced, i.e. the genotype frequencies in most of the separate nests just seem to reflect the genotype frequencies in the population as a whole. The most powerful mechanism to induce such relative homogeneity in eusocial Hymenoptera is a combination of polygyny and relaxation of colony-to-colony aggression, and in fact, the pattern of worker genotypes given in *table II* is very similar to genotype distributions reported for polygynous *Formica* ants by PAMILO (1982a) and PAMILO and ROSENGREN (1983, 1984).

If we compare *figure 1* and *table II*, it seems that deviating allele frequencies (all homozygotes in nest 24 and 28 and all heterozygotes in nest 37) occurred mostly towards the margins of the current distribution. As the deviations occur in both directions, this could perhaps reflect a lesser degree of polygyny, due to a rather recent colonisation of these boundary areas. In that case we should also expect to find more heterogeneous genotype-distributions at the recently colonised, but yet isolated spots in other parts of Budapest (see *fig. 1*, in VAN LOON *et al.*, 1990). Such irregularities are likely to be at least partly responsible for the fact that the correlation between genetic identity of nests and metric distance disappears if the larger metric distances are included in the analysis.

The data of *table III* suggest that single mating is the rule in *L. neglectus*. It is important to realize, however, that the chance to detect a second mating is relatively low from data of only one polymorphic marker-locus with two unequally represented alleles and involving small numbers of progeny. The precise chance of detection, calculated for the data in *table III* under the assumption of equal representation of paternal genotypes, was found to be 0.133 (see PAMILO, 1982b and VAN DER HAVE *et al.*, 1988 for methodology). Accordingly, if all eleven queens of *table III* would have been doubly inseminated, we would have expected to observe 1.2 double matings in this data set. The fact that not one double mating was observed suggests that it is unlikely that more than four of the queens of *table III* were doubly inseminated. We thus tentively conclude that single insemination prevails in *L. neglectus*.

The relatively high estimate for inbreeding and the low estimate for relatedness among nestmates deserve some further discussion. PAMILO (1985) derived equations which express worker-relatedness as a function of the inbreeding-coefficient, the frequency of intranidal mating and the number of (equally contributing) egg-laying queens. He concluded that relatedness among nestmate-workers is not much affected by intranidal matings if the nest sharing queens are unrelated, but that relatedness among worker-offspring increases more rapidly at increasing frequency of intranidal mating when queens arise as nestmates and are related to some extent. If we apply Pamilo's model which assumes nest-queens to be unrelated, there appears to be no solution that fits the conditions that both the frequency of intranidal mating ( $\alpha$ ) is  $\leq 1$  and that  $b'_{it}$  and  $F$  are within the 95% confidence limits of our combined estimates. The closest fit one can get is the combination  $\alpha = 1$ ,  $b'_{it} = 0.25$  and  $F = 0.10$ , which yields a mean number of queens of 3.2. Obviously, this scenario is unlikely to apply to the Budapest population of *L. neglectus*. It would require a massive nuptial flight in which only the queens participate, after being inseminated in their colonies of origin ( $\alpha = 1$ ). As reported by VAN LOON *et al.* (1990), it is precisely the apparent lack of a (queen-) nuptial flight which seems to characterize *L. neglectus*. We note, however, that this might not preclude all migration of queens, as it is always possible that mated queens walk to other neighbouring nests. There is also the — perhaps unlikely — possibility that some flights might occur at night.

We also applied PAMILO's (1985) model for related nest-queens to our empirical data. Assuming single insemination throughout, and using our estimated means and SE's for  $b'_{it}$  and  $F$ , it was possible to infer the relationship between the frequency of intranidal mating and the number of egg-laying queens per nest ( $n$ ). Our empirical results could not be fitted into this model when  $\alpha = 1$  ( $n$  becomes  $\infty$ ). However, as shown in *figure 2*, nearly exclusive intranidal mating would require hundreds of coexisting nest

queens, to end up with our estimates for offspring relatedness and inbreeding. If 50% of the males would fly off to mate elsewhere, the inferred number of nest-queens is still in the order of fifteen. In the unlikely case that the intranidal mating observed by VAN LOON et al. should appear to be a rare phenomenon after all, the inferred number of nest-queens comes out at around eight. As the assumption of this model — that queens arise as nestmates and have at least some relatedness towards each other — seems realistic for *L. neglectus*, we conclude that our estimates on relatedness and inbreeding can indeed be broadly explained by a combination of polygyny and intranidal mating. We note, however, that the PAMILO model used here does not take into account mixing of workers between nests. Thus, workers that were not born in the nest where they were sampled give no information on the number of queens in that particular nest. It seems likely that worker migration between nests will occur in *L. neglectus*, but the extent to which this might affect the inferred relationship of figure 2 remains to be seen.

As mentioned already in the introduction, one other polygynous species of the same subgenus, *L. sakagamii*, has been studied in Japan (YAMAUCHI

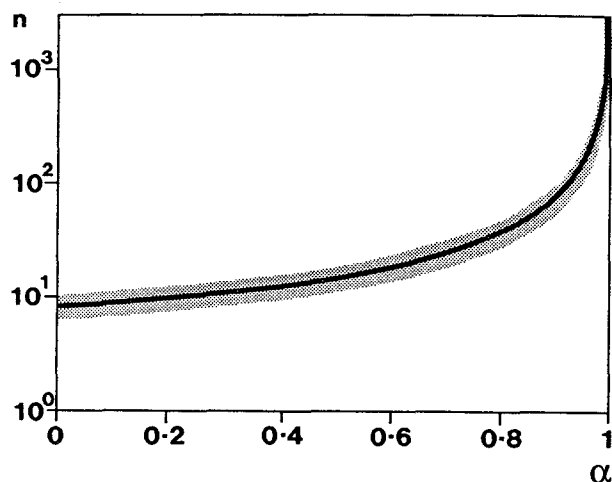


Fig. 2. — Inferred relationship between the number of nest-queens ( $n$ ) and the frequency of intranidal mating ( $\alpha$ ), according to the model for nest-mate (relatedness  $> 0$ ) queens by Pamilo (1985), applying the mean and SE of relatedness and inbreeding found for workers of *Lasius neglectus* ( $0.139 \pm 0.031$  and  $0.267 \pm 0.084$ , respectively). All nest-queens are assumed to be singly inseminated and to contribute equally to the offspring in the nest. The upper limit of the shaded "pseudo confidence region" was calculated by taking the mean + one SE for  $F$  and the mean — one SE for  $b'_{ff}$ . The lower limit applies to the reverse situation, i.e. the mean — one SE for  $F$  and the mean + one SE for  $b'_{ff}$ .

Fig. 2. — Dédution des relations entre le nombre de reines dans les nids ( $n$ ) et la fréquence des accouplements intranidaux ( $\alpha$ ) à partir du modèle proposé par Pamilo (1985) (coefficient de parenté  $> 0$ ). On a utilisé le coefficient de parenté moyenne et celui de consanguinité trouvé pour les ouvrières ( $0.139 \pm 0.031$  et  $0.267 \pm 0.084$  respectivement). On suppose que toutes les reines ne sont inséminées qu'une seule fois et contribuent de façon égale à la descendance du nid. La limite supérieure de la « pseudo-confiance » (zone hachurée) est donnée par la moyenne + une erreur standard pour  $F$  et la moyenne — une erreur standard pour  $b'_{ff}$ . La limite inférieure est donnée par la situation inverse, c'est-à-dire la moyenne — une erreur standard pour  $F$  et la moyenne + une erreur standard pour  $b'_{ff}$ .

and HAYASHIDA, 1970; YAMAUCHI *et al.*, 1981, 1982). Although no population genetical information has been collected for *L. sakagamii*, the available taxonomic and ecological data are detailed enough to make a comparison between the two species. YAMAUCHI *et al.* (1981) report the following characteristics for *L. sakagamii*: 1. intranidal mating, 2. polygyny through retention of newly emerged queens in the nest, 3. reduced territoriality, 4. colony multiplication by budding, 5. large colonies containing several millions of workers, 6. saturation and domination of the preferred local habitat. As far as data on *L. neglectus* are available by now, it seems that this new European ant species is very similar to *L. sakagamii* in most if not all of these six characteristics. It is unlikely, however, that *L. sakagamii* and *L. neglectus* are also very closely related (i.e. sister species). Not only are the two species very well separated geographically, there are also substantial differences in morphology and foraging behaviour (VAN LOON *et al.* 1990). A broader comparative analysis of allozymatic variation throughout the subgenus *Lasius* would probably shed more light on sister-species relationships and could also test the specific hypothesis of parallel evolution of similar polygyny-syndromes in separate lineages of the subgenus *Lasius*.

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