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Research article

Biological constraints and colony founding in the polygynous invasive ant *Lasius neglectus* (Hymenoptera, Formicidae)

X. Espadaler and S. Rey

C.R.E.A.F., Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain, e-mail: Xavier.Espadaler@uab.es

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Summary. The polygynous invasive ant *Lasius neglectus* was described from Budapest, Hungary, as an unicolonial species, with no apparent colony barriers, and inferred intranidal mating without a nuptial flight. Here we analyze additional morphological characteristics of gynes, their physiological condition at emergence and at the time of mating and we describe the productivity of different types of colony founding in the laboratory. A low increase in dry weight and in fat content from emergence to mating indicates that gynes can only succeed in dependent colony founding. However, the queen-worker thorax volume ratio is typical of a species with independent colony founding and we were able to demonstrate independent colony founding in the laboratory (both haplo- and pleometrotic). Brood development in independent founding is rapid and the number of nanitics higher than in other *Lasius* species. Both colony budding and dependent colony founding could also be demonstrated in the laboratory. Worker oviposition was absent. The carbohydrate content of newly mated queens is consistent with the observed loss of mating flight of this species. However the relative wing area clearly indicates that *L. neglectus* queens should be able to fly. Therefore, both queen morphology and physiological state at maturity show that *L. neglectus* is intermediate between a monogynous, free-living, non pest ant and a multiqueened (polygynic) invasive tramp ant. This neglected ant thus offers interesting opportunities to study the origin of unicoloniality and the spread of this species in northeast Spain.

Key words: Carbohydrate content, colony founding, egg-laying rate, fat content, *Lasius neglectus*.

Introduction

The degree of size dimorphism between queens and workers and the physiological condition of queens at emergence and at the time of mating are associated with the mode of colony founding (Hölldobler and Wilson, 1977). In independent colony founding the newly mated queen starts a new colony by herself, without the help of workers and she rears the first brood alone. Usually, such queens are claustral and do not forage during their confinement. In a few cases, however, the queen leaves the nest to forage, which has been referred to as non-claustral independent or semiclaustral colony founding. Alternatively, when a group of workers help the queen to found a colony (budding) or when new queens are accepted in the mother nest after mating (queen adoption), colony founding is dependent. This mode of reproduction is common in polydomous systems and some parasitic species.

In species employing independent claustral colony founding, the queens rely on their fat reserves and the proteins released and the energy provided by the histolysis of the flight-muscles (Janet, 1907). The relative fat content of such queens has been shown to be higher than in dependent founding queens (Keller and Passera, 1989). Stille (1996) showed a congruent difference in queen/worker thorax volume ratio between dependent and independent founding species, in that dependent founding queens had proportionally smaller ratios than independent founding queens.

A mating flight is usual in ants (Hölldobler and Wilson, 1990). Some species, however, do not have such a flight but mate directly inside the nest (some socially parasitic species) or in the vicinity of the nest (Buschinger, 1986; Felke and Buschinger, 1999). The glycogen content of ant sexuals appears to be related to their flight ability: non-flying species have a lower content of carbohydrates although their wing surface is not proportionately smaller than that of flying species (Passera and Keller, 1990).

Lasius neglectus is a poorly known species living in huge supercolonies with no apparent within-colony boundaries, and with a highly polygynous kin-structure (Van Loon et al., 1990; Boomsma et al., 1990). It has been considered an invasive pest ant by recent authors (Passera, 1994). Information concerning continental Europe (Seifert, 2000) and its status in Spain (Espadaler, 1999) indicate that this species has the potential of becoming a serious problem, similar to the argentine ant. We have undertaken a research program to characterize the basic biological characteristics of *L. neglectus* in Northeast Spain. Here we examine the physiological status and a number of morphological characteristics of the gynes of *Lasius neglectus* at the time of mating to ascertain whether these data indicate a dependent or an independent colony founding and the presence or absence of a mating flight. Specifically, we have determined the relative fat content of queens and the queen/worker thorax volume ratio to have a good approximation of the most likely mode of colony founding. We have further used the carbohydrate content and the relative wing area to obtain an independent estimate of the likelihood of mating flight in *Lasius neglectus*. Finally, we have tested the success of dependent and independent founding in the laboratory and we have determined worker egg-laying capacity.

Table 1. Presence of castes, sexes and developmental stages of *Lasius neglectus* in the population of Seva, Barcelona (data from April 1999 to November 2000)

Month	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Eggs				X	X	X	X	X	X	X		
Larvae	X	X	X	X	X	X	X	X	X	X	X	X
Worker pupae					X	X			X	X		
Sexual pupae					X	X						
Young workers					X	X				X	X	
Sexuals					X	X						

Materials and methods

The species and colony used in the analysis

Lasius neglectus was recently described from Budapest, Hungary (Van Loon et al., 1990) and further data on distribution are provided by Seifert (2000). The huge, highly polygynous colony of *L. neglectus* studied here occupies at present a continuous surface of 14 hectares in a suburban zone in northeast Spain, at 650 m a.s.l. The climate is Mediterranean, with a wet spring and fall and a dry winter and summer. Mean annual temperature is 11.5 °C and annual rainfall ranges from 700 to 750 mm. Throughout the area colonized, queens are found isolated or in small group (2–5 queens) under medium-sized stones. Houses are widely spaced and many lots have so far remained without human construction and have a mildly degraded natural vegetation. Irrigated gardens have both native trees and exotic bushes and trees. Monthly routine checks of life stages (eggs, larvae, pupae and adults) were performed during the activity period (April 1999 to November 2000). The life history for this colony is summarized in Table 1. Polygyny was ascertained through queen dissection and looking for a full spermatheca and developed ovaries. Within the limits of the 14 ha colony, the area is exclusively occupied by *L. neglectus* (pers. obs.). Two other populations have been reported in north-east Spain (Espadaler, 1999) and four more have since been detected (see Table 2). So far, *Lasius neglectus* has never been observed in natural habitats in Spain but only in disturbed areas from where the original vegetation has been cleared, substituted or heavily modified. B. Seifert (pers. comm.) is currently updating the distribution of the species. In four of the seven populations polygyny has been ascertained. The pest status of this ant is clear from the first reports (Boomsma et al., 1990; Tartally, 2000). In Spain, affected inhabitants of private property invaded by *Lasius neglectus* report extensive presence inside homes, occupation of electrical conduction with damage to electrical outlets, phones and electric swimming pool engines. Outside, ant aphid-attendance, in enormous numbers has caused the death of some of the affected trees. The ants were first detected, merely as a mild nuisance, in 1985.

Determination of fat content, queen/worker thorax volume ratio, carbohydrate storage and relative wing area

To measure the lipid content of queens we followed the same protocol as Keller and Passera (1989), based on Peakin (1972). In short, individual queens were weighed fresh, then dried for 24 h at 70 °C and the dry weight determined. Petroleum ether (boiling point 40–60 °C) and a Soxhlet apparatus were used to extract fat during 24 h from queens, which were subsequently dried again and weighed to the nearest 10^{-4} g, to obtain the lean weight. Fat content is expressed as a % of dry weight. Fat content was measured from queens at emergence (n = 24) and from queens at the time of mating (two weeks old; n = 10). Mating was observed at the laboratory and newly inseminated queens were used. Throughout the paper means are presented ± s.d. All statistical tests are two-tailed.

Queen/worker thorax volume ratio was determined as in Stille (1996). Thorax volume was estimated as length × width × height. We used a mean of two-three queens and three workers each from five distinct populations.

Carbohydrates (glycogen and free sugars) were determined following Van Handel (1985) and Passera and Keller (1990) with modifications to optimize the procedure. Queens were dried at 90 °C for 45 min., weighted and analyzed individually. A polytron was used, for a minimum of 30 s, to crush and homogenize each ant in water (400 µl per ant). The homogenate was centrifuged (30 s at 1500 rpm) in a bench top microfuge to eliminate the exoskeletal chitin to avoid interference in spectrophotometric analysis. Only the supernatant was used to analyze the carbohydrate content. Two replicates for each sample (250 µl of supernatant for each replicate) were obtained. Subsequently, the protocol of Van Handel (1985) was followed utilizing a Spinvac centrifuge (2 h at 50 °C) to evaporate the methanol. Anthrone (Sigma A1631) was

Table 2. Geographical and climatic characteristics of several known populations of *Lasius neglectus* in Northeast Spain

Locality	Longitude/Latitude	Elevation (m)	Rainfall*	Temperature**
Seva	41°48′N, 2°16′E	650	775	11.5
Bellaterra	41°30′N, 2°6′E	90	675	14.5
Barcelona	41°23′N, 2°9′E	20	575	16.5
San Cugat	41°28’N, 2°5’E	130	725	13.5
Les Planes	41°26′N, 2°6′E	230	725	14.5
Matadepera	41°37′N, 2°2’E	570	775	13.5
Taradell	41°53′N, 2°18′E	650	775	13.5

* Mean annual precipitation (mm.), ** Mean annual temperature (°C).
Extracted from Atles Climàtic de Catalunya. Termopluviometria (1997). Institut Cartogràfic de Catalunya (www.icc.es).

used as the color reagent. Optimal wavelength for spectrophotometric analysis of the green-blue reaction product is 625 nm. Optical Density (OD) was determined, and glycogen and sugar content read directly from the glucose calibration line.

Surface area of the wings was determined as in Passera and Keller (1990) by sketching the wing profiles with a drawing tube, cutting the profile, weighing the cuts and inferring the wing area from the weight of a known area of the same paper. This area was subsequently related to the fresh weight.

Testing independent colony founding

Queens and males were captured in the field in June. In the laboratory they were allowed and observed to mate freely in a large plastic box. On the following morning, dealate queens were assumed to be mated. Twenty replicates of single queens (haplometrotic) and twenty of pairs of queens (pleometrotic), without workers, were placed in vials (7 cm length, 1 cm diameter) with a water reservoir behind a cotton plug and no food. Egg-laying rates were obtained by a daily count of eggs during the first three days. Thereafter, vials were checked two or three times per week for the appearance of larvae, pupae and up to the eclosion of the first nanitic workers. The total number of workers produced (dead + alive) were counted until the original field colony started hibernation (November). Laboratory temperatures ranged from 22.5 °C minimum at night to 30 °C during daytime.

Testing colony budding and dependent colony founding

We use colony budding in the sense of Bourke and Franks (1995): ”colony foundation by a queen (or queens) plus workers leaving an established colony”, in which the age of queens is not defined. Old queens were captured in the field before the appearance of the new queen generation in June (Table 1). As the number of attending workers may influence the output of queens and the rearing of the new brood (Keller and Passera, 1990; Cassill and Tschinkel, 1999), two nest compositions were used. Single queens were placed with either 30 or 60 workers in vials (n = 5 replicates each) and fed with Bhatkar's artificial diet (Bhatkar and Whitcomb, 1970) once a week. Egg-laying rates were recorded as before and vials were maintained until the appearance of pupae and workers. Dependent colony founding was not systematically studied but for three artificial nests with >15 freshly mated queens and >100 workers. Egg laying and larval development until workers eclosion was ascertained.

Testing worker oviposition

Male production by worker ants has been reported for *Lasius niger* (Van der Have et al., 1988). To test the possibility of worker egg-laying, two groups of 100 workers older than seven months and two groups of ca. 50 worker pupae plus 100 old workers were established to check for possible egg-laying in old or in young, freshly eclosed workers. Also these colonies were maintained in vials, and fed Bhatkar's artificial diet twice a week. Freshly eclosed workers of one, two and three weeks old and old workers were dissected in Ringer' insect solution to study ovary development. The stage of development of the ovarioles and the presence of yellow bodies was examined under the microscope.

Results

Fat content, body-size relationship and carbohydrate content

The mean fat content of queens at the time of mating was of 43% (Table 3). Relative fat content of queens showed an increase of 46% from emergence to mating with an increase in total dry weight of 55%. Queen and worker thorax volumes were 3.62 ± 0.24 and 0.15 ± 0.19 mm^3 respectively. The mean queen/worker thorax volume ratio obtained for five populations of *Lasius neglectus* was 23.83 ± 3.59.

Table 3. Physiological status (mean ± SD) of *Lasius neglectus* gynes at the respective times of emergence and mating. Data are based on one Iberian population (Seva, Barcelona)

	Dry weight (mg) (24 h at 70 °C)	Fat content (%)
Emergence	3.1 ± 0.5 (n = 23)	29.6 ± 9.7 (n = 23)
Mating	4.8 ± 1.0 (n = 10)	43.3 ± 9.5 (n = 10)
Increase (%)	54.8	46.2

Glycogen content of mature queens was 225 ± 92.2 µg, and the weight of free sugars was 69.2 ± 20.8 µg. The mean dry weight of *Lasius neglectus* mature queens after 45 min at 90 ºC was 5.8 ± 1.2 mg (n = 10). Therefore the values of the glycogen and free sugars as percentage of dry weight were 3.84% and 1.26%, respectively. The amount of total carbohydrates (glycogen + free sugars) expressed as percentage of dry weight was 5.1 ± 1.2%.

The forewing area of gynes was of 20.38 ± 0.68 mm^2 (n = 7) and the ratio of wing area to body fresh weight (mean fresh weight at mating: 11.35 mg) was 1.74 ± 0.15.

Independent colony founding

Lasius neglectus queens are able to found new colonies independently. In almost all foundations (38 of 40), both pleometrotic and haplometrotic, egg laying began on the first day. The egg laying rates for young queens founding independently in laboratory nests were 6.8 ± 2.4 eggs/queen/day (n = 20; single queen) and 6.1 ± 3.0 eggs/queen/day (n = 20; two queens). Egg-laying rates did not differ between neither haplo- and pleometrotic foundations (ANOVA, $F_{1,38}$ = 0.6; P = 0.4) nor was there any difference in duration of egg, larval and pupal stages (MANOVA, Wilks' Lambda = 0.98; P = 0.95). The mean time of egg incubation was 12.1 ± 2.3 days; larval development lasted for a mean of 9.3 ± 2.1 days and the pupal maturation was 14.5 ± 2.5 days. The total development time from egg to a nanitic worker in *L. neglectus* was 36 ± 4 days. The mean egg size was 0.55 × 0.33 mm (n = 21).

After four months (from 13/6/00 to 9/10/00) haplometrotic foundations (n = 20) produced 11.5 ± 4.8 nanitics and pleometrotic foundations (n = 16) produced 19.2 ± 7.5 nanitics. Totals were different (ANOVA, $F_{1,34}$ = 14.6; $P < 0.001$) but no differences were found between the rates of nanitics produced per queen (11.5 ± 4.8 vs. 9.6 ± 3.7) for the two modes of colony founding (ANOVA, $F_{1,34}$ = 1.2; P = 0.26). Mortality before the appearance of the first workers was 2/20 (haplometrotic) and 1/20 (pleometrotic). No aggression was ever detected between pleometrotic queens before or after the appearance of nanitics.

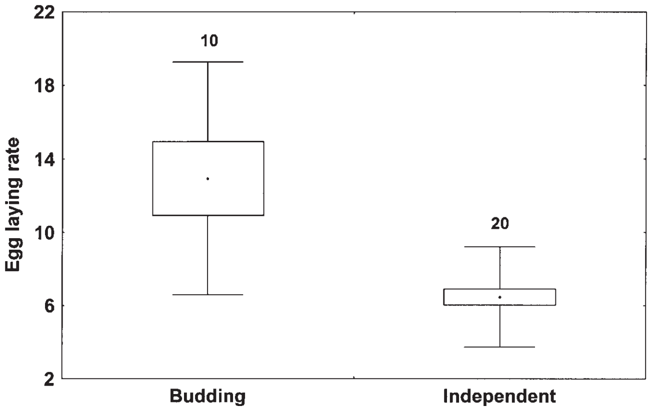

Figure 1. Egg laying rates (mean number of eggs/day/queen; three days) produced by old queens + workers (budding) and independent founding queens of *Lasius neglectus* in laboratory nests. Central point: mean; box: S.E.; whiskers: S.D. Number of replicates is given above each box

Colony budding and dependent colony founding

Although the number of workers between the nests differed (30 or 60) there were no differences in egg laying rate between treatments (ANOVA, $F_{1,8} = 0.0039$; $P = 0.951$). The egg-laying rate for old queens was 12.9 ± 6.3 eggs/queen/day (n = 10). All nests contained only one old queen.

The egg-laying rate for old queens is significantly higher than the egg-laying rate for the independent founding young queens (ANOVA, $F_{1,54} = 25.66$; $P < 0.001$; Fig. 1). The mean time of egg incubation was 31.2 ± 8.8 days (n = 10); larval development lasted for a mean of 26.4 ± 2.5 days (n = 5) and the maturation of the nymph lasted for a mean of 13.3 ± 1.1 days (n = 3). The mean development time from egg to worker in eggs laid by old queens was 70.9 days, much longer than in the independent foundations (MANOVA, Wilks' Lambda = 0.15; $P < 0.001$). Queen mortality of those >1 year old queens, before new workers had eclosed was three out of ten, a higher mortality than in independent foundations (three out of 40; <1 year old queens). This difference approached significancy (p = 0.056; difference between two proportions). Pupae and young workers appeared in the three artificial nests simulating dependent founding.

Worker oviposition

Worker dissection showed that young workers have two developed ovarioles. In workers of 5 to 10 days old, one egg was usually present in one of the ovarioles. Its aspect, however, was rounded and no defined chorion was ever seen. The size of the meroistic ovarioles ranged from 1 to 1.60 mm length. The size of the developed egg was 0.4 × 0.3 mm. Older workers had regressed or completely degenerate ovarioles (0.5 to 0.8 mm length) with 2 yellow bodies present (n = 25). After 4 months the ovarioles always appeared completely degenerate, similar in aspect to those described in *Formica* (Billen, 1984), *Myrmica* (Minkenberg and Petit, 1985) or *Ectatomma* (Fénéron and Billen, 1996). No eggs ever appeared in nests of old workers nor in nest of young + old workers during the two months of observation. Worker egg laying is thus probably restricted to the production of relatively few trophic eggs.

Discussion

The physiological status of *L. neglectus* gynes at emergence and at mating time shows a puzzling mixture of characteristics. The increase in total dry weight from emergence to mating and the relative fat content at mating of young queens of *Lasius neglectus* falls within the range of data obtained for ants with a dependent mode of colony founding (Keller and Passera, 1989). The values of relative fat content at mating (43.3%) are intermediate between the values for independent and dependent colony founding species. The increase in fat content from emergence to mating time (46.2%) is definitely within the values for the dependent founding species.

In contrast, thorax-volume ratio of queens to workers (23.8) shows that *L. neglectus* has still retained the morphology corresponding to independent colony founding. The ratios obtained for other *Lasius* species (Stille, 1996) based on the type of foundation were very distinct: on independent founding species, ratios varied between 24.1 for *L. niger* and 31.6 for *L. psammophilus* Seifert. For the dependent or temporary parasitic *Lasius* species, thorax ratios were from 2.0 to 9.5. In some other genera the division into groups of dependently and independently founding species was not as distinct as in *Lasius* (Stille, 1996). In the population studied we have not checked the intranidal mating in the wild and probable subsequent budding process, that has been attributed to this species (Van Loon et al., 1990; Boomsma et al., 1990). At the population studied, inhabitants have never detected masses of sexuals at swimming pools in spite of those swimming pools being surrounded by lawns invaded, exclusively, by *L. neglectus*. Boomsma et al. (1990) inferred that *L. neglectus* has intranidal mating; as we have observed matings in boxes, without a nuptial flight, this is an additional evidence for intranidal mating. If we could eventually confirm intranidal mating at the field, it would imply that *L. neglectus* has the best of both worlds: young queens have still the relative large thorax typical for independent founding species but, instead, the workers adopt them. However, the increase in fat content from emergence to mating time shows clearly that *L. neglectus* gynes have initiated the evolutionary development towards dependent founding.

With regard to the total carbohydrate content (5.1 ± 1.2%) *Lasius neglectus* gynes have an intermediate value between the flying (7.7 ± 2.2%) and the non-flying (2.8 ± 0.9%) gynes (Passera and Keller, 1990). Glycogen content was far higher than that of free sugar in *Lasius neglectus* gynes. Wing area development in relation to fresh weight of *L. neglectus* gynes is similar to values obtained for the gynes of a non-flying species, *Linepithema humile* (1.70 ± 0.13) by Passera and Keller (1990). By its wings *L. neglectus* does not show any impediment to be able to make a mating flight.

This mating flight has never been observed in nature for this species and is probably absent (Van Loon et al., 1990; but see Seifert, 2000). Independent colony founding is possible by both single queens and groups of *L. neglectus* queens in the laboratory; thus, a propagule size of 1 young, fertilized queen is sufficient for colony initiation. This was already stated in short by Boomsma et al. (1990). Apparently, mated queens of *L. neglectus* have enough body reserves to lay eggs and rear the first young without any external food during four weeks. Boomsma et al. (1990) suggested a lesser degree of polygyny towards the edges of the distribution; it is likely that it is mostly there that independent foundings take place in the field. That pleometrotic foundations produce a larger initial brood in *L. neglectus* is not unexpected, as this is well documented for other species (refs. in Sommer and Hölldobler, 1995). Colony budding in the form of old queens with the help of workers, were also able to start new colonies and their egg-laying rate was higher than that of young queens. In this last aspect, *L. neglectus* behaves more like an independent founding species than a dependent founding species. Keller and Passera (1990) showed that queen fecundity was higher in old queens of independent founding species (*Lasius niger, Camponotus herculeanus, C. ligniperda*) but was similar for both old and young queens in dependent founding species (*Plagiolepis pygmaea, Linepithema humile*). As could be anticipated, dependent colony founding of young queens with workers is also successful in *L. neglectus*. The absence of reproductive egg laying in workers shows also an evolution towards the tramp species life history, one of whose characteristics is this absence of worker oviposition (Passera, 1994).

According to our laboratory results, that nanitics produced by isolated queens develop in roughly a month, we should expect to find pupae during July and August. However, we did not observe pupae during those months in the field (Table 1); instead, it seems that there are two distinct cohorts of workers. A possible explanation may be that there is an arrest of egg eclosion and/or larval development (due to high temperature?); this is to be ascertained by mantaining brood under different temperatures at the laboratory. Alternatively, the food provided to larvae of recently mated queens could be different from that given to larvae of mature queens, which might, in turn, influence the rate of brood development. To understand the absence of worker pupae in the mid of summer, more work is required and should be confirmed in other populations. The development time of nanitics, from young queens, was much shorter than that of normal size workers from old queens. A short development time of the immature brood has been invoked as a factor intervening in the rapid spread of a social insect, *Polistes dominulus* (Cervo et al., 2000); although not directly comparable, this could be among the factors promoting the invasiveness of *L. neglectus.*

Polygyny is expected to result in the reduction of fecundity per queen (Tsuji and Tsuji, 1996). Although the per queen output (eggs to nanitics) in *L. neglectus* was higher in haplometrotic foundations neither the egg laying rates nor the number of nanitics per queen were statistically different. Brood development time in *L. neglectus* was shorter than in other *Lasius* (s.str.) species. In *L. niger*, the first eggs, larvae and pupae appeared later than in *L. neglectus*: in the 5th week (Keller and Passera, 1990) or after a mean of 8 weeks (Sommer and Hölldobler, 1995). A *L. alienus* queen laid a first egg one month after the mating flight (Černá, 1978) and for *L. pallitarsis* the first workers emerged after 13 weeks (Nonacs, 1990). The number of first workers also seems to be higher than in other *Lasius* (s.str.) species: 3 workers/queen after 60 days in *L. niger* (Sommer and Hölldobler, 1995) or one worker/queen after 17 weeks in *L. pallitarsis* (Nonacs, 1990). Those comparisons are not quite accurate as much of it depends on the temperature and different authors may not have reared their queens at the same temperature. It remains to be proved if solitary or grouped queens can found new colonies in the field.

A single other species of *Lasius* (s.str), *L. sakagamii*, from Japan, is known to be polygynous (Yamauchi et al., 1981). This seems to indicate two independent origins of unicoloniality in *Lasius* (s. str.). Otherwise, it seems that these evolutions towards high polygyny are rare in *Lasius*, compared to other genera (e.g. *Formica*). Our results indicate that *L. neglectus* evolved rather recently, because many adaptive behaviours for polygyny are in place, but the morphology has not yet followed. In short, *L. neglectus* has a series of highly interesting characteristics as an evolutionary intermediate between the standard *Lasius* type and a real unicolonial ant such as the argentine ant. In view of its dangerous pest potential we emphasize the urgent need for a careful identification of suspected colonies, perhaps wrongly attributed to other *Lasius* species and of biological studies directed towards a thorough knowledge of the biology of *Lasius neglectus*.

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