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THE PHENOMENON OF A MIXED COLONY: THE CASE OF *LASIUS BRUNNEUS* AND *LASIUS UMBRATUS* (HYMENOPTERA, FORMICIDAE)

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The Phenomenon of a Mixed Colony: the Case of *Lasius brunneus* and *Lasius umbratus* (Hymenoptera, Formicidae). Stukalyuk, S., Radchenko, Yu., Akhmedov, A. & Stelia, V. — The aim of this work is to study to find out why, in the case of temporary social parasitism, a mixed colony of *Lasius brunneus* (Latreille, 1798) and *Lasius umbratus* (Nylander, 1846) exists in this state for many years. In 2023, a mixed colony of *L. brunneus* (host species) / *L. umbratus* (temporary social parasite) was discovered in Moldova. This mixed colony is located at the same site where another mixed colony containing *Lasius fuliginosus* (Latreille, 1798), *L. umbratus* disappeared in 2017. Video recordings of the intensity of movement of workers of the two species along the trails were made to study the frequency of antennal contacts and to calculate the colony population. Based on the previously used algorithms, it was assumed that the queen oviposition rate of *L. umbratus* is 1.5 times higher than that of *L. niger*, while that of *L. brunneus* is comparable to that of *Lasius niger* (Linnaeus, 1758). Chi-square test and post-hoc Dunn's test were used for statistical analysis of the data. Mathematical modelling was used to analyse the rate

of colony development. Results of the analysis of worker activity on the trail showed that *L. brunneus* had 11 times more foragers than *L. umbratus*. A high number of interspecific antennal contacts between workers was recorded. The population of the mixed colony was estimated to be 48.880 *L. brunneus* and 24.433 *L. umbratus* workers. Mathematical modelling confirmed the failure of *L. brunneus* monogyny in a mixed colony and suggested that high *L. brunneus* abundance is ensured by multiple queens (oligogyny). Probably in 2021, the *L. brunneus* colony moved to a new habitat, which was invaded by the *L. umbratus* queen. After the invasion, several *L. brunneus* queens survived, allowing a mixed colony to exist.
Key words: *Lasius umbratus*, *L. brunneus*, monogyny, oligogyny, mixed colony, mathematical modelling.

Introduction

Mixed colonies are quite common among ants. They are known among red wood ants, for example between *Formica aquilonia* Yarrow, 1955, *F. polyctena* Foerster, 1850 and *F. rufa* Linnaeus, 1761 (Czechowski & Radchenko, 2006; Korczynska et al., 2010), and between *Formica pratensis* Retzius 1783 / *F. cinerea* Mayr, 1853 and *F. clara* Forel, 1886 (Czechowski & Radchenko, 2007). There are also known cases of mixed colonies of *Lasius* and *Myrmica* species, *Lasius* and *Leptothorax*, although all these cases belong to compound nests (Antonova, 2004).

In mixed colonies, one of the species is usually a temporary social parasite. The queen of the temporary social parasite species, after entering the nest, kills the queen of the host species and takes her place. Workers of the temporary social parasite species eventually replace workers of the host species. *Lasius umbratus* establishes colonies at the colonies of *L. niger* / *L. brunneus*, *L. fuliginosus* — at the colonies of *L. umbratus* (Seifert, 2018), When the population of the host species becomes extinct (because there is no replacement due to the death of the queen), the mixed colony ceases to exist. Previously, mixed colonies of *L. fuliginosus* / *L. umbratus* have been recorded (Donisthorpe, 1915; Lodeizen, 1946; Furukawa, 1953; Seifert, 2018; Stukalyuk et al., 2021), and, in some cases, existence of such colonies for at least 4 to 6 years was recorded (Stukalyuk et al., 2022). During this period of coexistence, the population of the host species should normally have been completely extirpated within 1 or 2 years. *Lasius niger* workers are known to live for 309 to 434 days, depending on the colony size (Kramer et al., 2016). Cases where a mixed colony exists for more than 2 years are relatively rare (4 mixed colonies were found in Ukraine and Moldova, Stukalyuk et al., 2022), and the colonies found are mainly associated with urbanised areas.

In 2023, we discovered for the first time a mixed colony of *Lasius brunneus* / *L. umbratus*. The average colony size of *L. brunneus* is 74 thousand workers (maximum 200 thousand, Stukalyuk et al., 2022), for *L. umbratus* — from 20 to 70 thousand. The uniqueness of the discovered mixed colony lies in the fact that it occurred in the same place where a mixed colony of *L. umbratus* / *L. fuliginosus* occurred between 2012 and 2017. From a mathematical point of view, the probability of a mixed colony reappearing in the same place as the previous one is very low. This emphasises uniqueness of the case studied, namely the founding of a mixed colony at the same place, where previous mixed colony was exist earlier. We considered two hypotheses for the founding and growth of a new colony (in a dependence from location of founded by *L. brunneus* colony): a) foundation of the colony first by *L. brunneus* on this site, then the invasion of *L. umbratus*; b) relocation of the *L. brunneus* colony from another site; subsequently, at its expense, a colony of *L. umbratus* was established at a new site. Analysis of this data can contribute to a better understanding of the biology of parasitic ant species, as well as to the clarification of incompletely studied features of their activity.

The aim of this work is to study to find out why, in the case of temporary social parasitism, a mixed colony exists in this state for many years. Objectives: a) study activity of two species on the trail; b) calculate the population of a mixed colony; c) compare the population data obtained with the results of mathematical modelling of the colony growth.

Material and Methods

The study was carried out in June 2023 in the territory of Moldova, near the town of Rybnitsa (coordinates 47.65′09″82, 29.09′87″23, summer cottage). A mixed colony of *Lasius brunneus*, *L. umbratus* was studied, founded in the same place where a mixed colony of *Lasius fuliginosus*, *L. umbratus* existed here between 2012 and 2017 (Stukalyuk et al., 2021). The nest was located at the base of a walnut tree (*Juglans regia*) of 0.4 m diameter. In 2023, a foraging trail was discovered on the tree trunk along which *Lasius brunneus*, *L. umbratus* workers used to move. Ants of both species emerged from a hole in the trunk at a height of approximately 0.6 m from the ground surface. We did not excavate the nest, however, given that *L. brunneus* is a dendrobiont, it is likely that most of the nest was originally located in the lower part of the tree trunk.

In order to analyse activity of the workers on the trail and contacts between them, a video recording was made. The camera was mounted on a tripod and filmed at an angle of 90 degrees to the trail. Filming was carried out on the same section of trail. A total of 4 videos 4 minutes each were recorded. Trajectories of 307 *Lasius brunneus* workers and 28 *L. umbratus* workers were considered. For each ant, the number of interspecies and intraspecies antennal contacts made within 2 seconds was analysed. The number of ants that did not participate in antennal contacts and simply followed the trail in both directions was also taken into account. In total, 317 antennal contacts within 2 seconds were analysed for both species and 877 cases where workers did not participate in contacts. Video analysis was carried out using the Movavi video editor.

We then counted the number of workers of each species passing along the trail within 1 minute; a total of 16 such counts were made. This is necessary to calculate the population of a mixed colony according to the A. A. Zakharov formula (1978, 2015), which is suitable for all ant species with foraging trails. The formula consists of 2 parts, the first calculates the number of foragers, the second — total population of the colony.

(1) $A = 36.82 - 2.127\ I + 0.112\ I^2 - 0.00047\ I^3$

A — number of foragers (hundreds of workers); I — number of workers (workers per minute on the trail). Precision of the method is P = ± 1.6 %.

(2) $N = 7.7A$

Mathematical modelling

In calculating the colony growth rate, we used the same algorithms as in our other work describing the mixed colony of *L. umbratus* / *L. fuliginosus* (Stukalyuk et al., 2021). The queen oviposition rate of *L. umbratus* was assumed to be 1.5 times higher than that of *L. niger*, while that of *L. brunneus* was comparable to that of *L. niger*.

Based on the assumption that if we had not already recorded a mixed colony of *L. umbratus* / *L. fuliginosus* in 2017, a colony of *L. brunneus* could have been founded at this site even then. We considered two hypotheses: 1) establishment of a *L. brunneus* colony in 2017 and introduction of a *L. umbratus* queen into it in 2020; 2) relocation of an already large *L. brunneus* colony to a new habitat in 2020 and introduction of a queen *L. umbratus* into this colony in the same year.

We have made an assumption that there may be more than one queen in a *L. brunneus* colony, otherwise it does not seem possible to explain its large size (about 50 thousand workers). According to our calculations, for a colony with one queen, the *L. brunneus* colony would reach a maximum of 16,000 workers by 2023 (fig. 1). Therefore, if there was only one queen in the *L. brunneus* colony, the colony would not have time to grow to this size in 6 years.

Statistical analysis

The data was analysed using Past (v. 4.13) software. The chi-squared test was used to identify significant differences between the parameters compared (number of workers on the trail during the 2-minute count, number of antennal contacts during the 2-second count, number of workers not involved in antennal contacts during the 2-second count). The post-hoc Dunn's test (p-value, z-value) was used to identify significant differences within each of these parameters. The data obtained from the statistical analysis are presented in tables and graphs (violin and box plots).

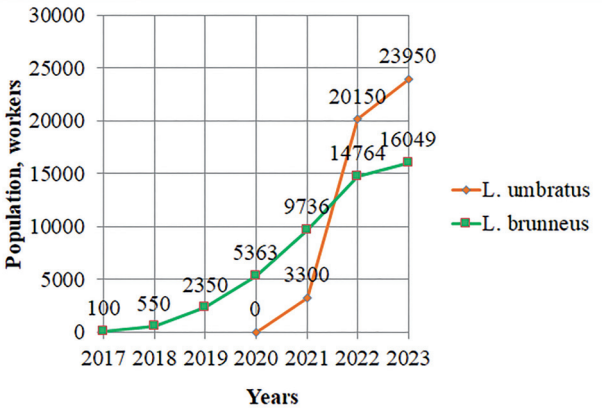

Fig. 1. Calculated growth dynamics of a mixed colony with only one *L. brunneus* queen.

Results

Number of foragers on the trail

It was found that the number of *L. brunneus* foragers on the trail was 11 times higher than that of *L. umbratus* (fig. 2, table 1).

It is interesting to note that *L. umbratus* workers, despite their underground lifestyle, left the nest as part of a mixed colony and moved in both directions along the trail. *L. umbratus* thus changed its lifestyle and foraged on the surface.

Number of antennal contacts

A consequence of different numbers of workers on the trail is a different number of antennal contacts (fig. 3, A; table 1).

Despite the lower number of workers on the trail, *L. umbratus* workers had significantly more contacts with *L. brunneus* workers than with each other. The number of contacts between *L. brunneus* workers is maximum, which can be explained by the high number of workers on the trail. This indicates the active participation of *L. umbratus* workers in both intraspecific and interspecific contacts. The number of *L. brunneus* workers without antennal contact was on average 15 times higher than the number of *L. umbratus* workers (fig. 3, B; table 1).

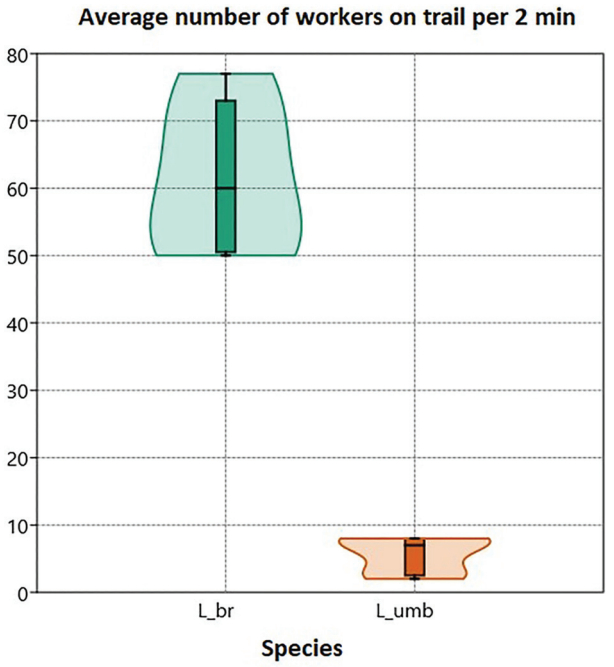

Fig. 2. Average number of *Lasius brunneus* and *L. umbratus* foragers moving on the trail during a 2-minute count.

Population of the mixed colony

The calculations have shown that the population of the mixed colony was 48,880 *L. brunneus* and 24,433 *L. umbratus* workers. The total number of foragers on the trail also differed by a factor of ca. 2 : 6348 *L. brunneus*, 3173 *L. umbratus*. In order to find out how a colony with such a composition could develop, we carried out a mathematical modelling.

Table 1. Results of statistical analysis for activity parameters of *Lasius brunneus* and *L. umbratus* workers on the trail of a mixed colony

Parameter	Chi-square	DF	Z	p-value
# of *L. brunneus* workers vs # of *L. umbratus* workers on trail / 2 min	6.818	9	2.619	0.008
# of antennal contacts: *L. umbratus-brunneus* vs # umbratus-umbratus / 2 min	116.3	1368	5.034	≤ 0.0001
# of antennal contacts of *L. umbratus-brunneus* vs # *L. brunneus-brunneus* / 2 min			10.44	≤ 0.0001
# of antennal contacts of *L. umbratus-umbratus* vs # of antennal contacts of *L. brunneus-brunneus* / 2 min			15.47	≤ 0.0001
# of workers not involved in antennal contacts / 2 min	220.7	913	16.07	≤ 0.0001

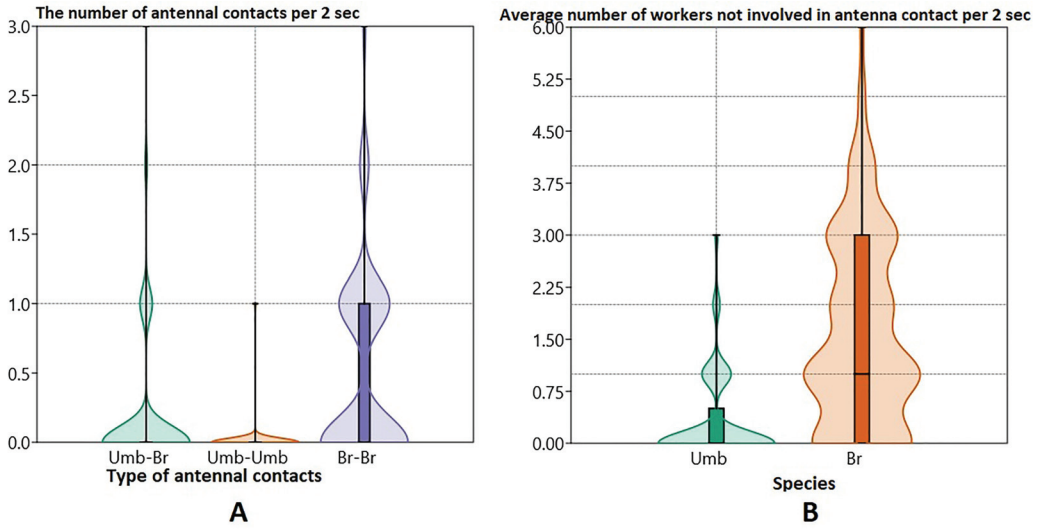

Fig. 3. A — average number of antennal contacts between workers on the trail in 2 seconds (Umb — *L. umbratus*, Br — *L. brunneus*); B — average number of workers not involved in antennal contacts.

Taking into account the assumption of presence of several queens in *L. brunneus*, we considered two main hypotheses that suggest presence of several queens in *L. brunneus* at the beginning of the colony and until the invasion of *L. umbratus* (fig. 4, A, B).

In both cases, development of the *L. umbratus* colony took 3 years to reach 28 thousand workers. For *L. brunneus*, the difference is not in the rate of development, but in the change of habitat location. We assumed that once high abundance was reached, the *L. brunneus* colony would remain at approximately the same level, maintained by several queens. Development of the *L. umbratus* colony was only possible with invasion of the queen into the *L. brunneus* colony. If we assume that after the death of the *L. fuliginosus* / *L. umbratus* mixed colony in 2017, the latter remained, then unification into a mixed colony with *L. brunneus* would be impossible due to hostile relations between workers of already adult independent colonies.

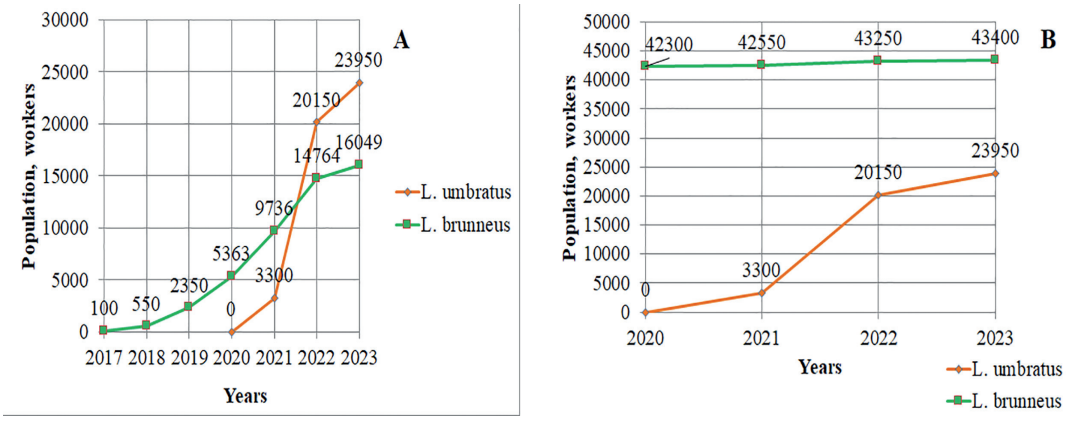

Fig. 4. Calculations of growth dynamics of a mixed colony according to hypotheses 1 (A) and 2 (B).

Discussion

Despite the fact that *L. brunneus* is characterised by monogyny, Seifert acknowledges existence of oligogyne colonies (Seifert, 2018), so our assumption of multiple queens can be hypothetically tested. For *L. umbratus*, it also allows for oligogyny through pleometrosis. We believe that presence of one or more *L. umbratus* queens will only change the colony growth rate, but presence of one queen of this species is accepted as the base case. If a *L. umbratus* queen invades a *L. brunneus* colony, only one queen of this species may die and 2 or 3 others, located in different parts of the nest, may remain alive. This mechanism may ensure existence of a mixed colony.

L. umbratus workers were only discovered on the surface in 2023, i. e. before that the development of their part of the mixed colony took place underground. Previously, only *L. brunneus* workers had been observed on the surface at this site for 2 years.

Another argument for the survival of several *L. brunneus* queens is the population size of the species in a mixed colony. To reach a size of about 30 thousand workers, *L. umbratus* would need at least 3 years. During this time, the *L. brunneus* population should have become extinct instead of remaining at almost 50 thousand workers. According to the literature, complete disappearance of workers of the host species occurs 14 months after the invasion of the *L. umbratus* queen, i.e. in half the time (Crawley, 1909; Holldobler, 1953). In some cases, *L. fuliginosus* workers have killed *L. umbratus* workers in a mixed colony (Lodeizen, 1946). The ratio of the mixed colony population of 1:2 in favour of the host species shows the opposite situation in relation to parasitic species. Thus, for *Lasius jensi* (Seifert, 1982), there are only 100 workers of the host species *Lasius alienus* (Foerster, 1850) for its 800 workers 14 months after the establishment of the colony, i. e. 1 : 8 (Seifert, 2018). In the mixed colonies of *L. umbratus* / *L. fuliginosus* that we previously discovered, the ratio was approximately 1 : 2, 1 : 3 in favour of the superparasite *L. fuliginosus* (Stukalyuk et al., 2021). Therefore, in mixed colonies the population ratio is usually in favour of the parasitic species; the host species is numerically dominant only during the first 2 or 3 years of the colony's life. The colonies we studied were 4 to 6 years old. In addition, a ratio in favour of the parasite is possible if the number of its queens is greater than one, if a constant population growth is ensured. It is known that in *L. fuliginosus*, which also has physogastric queens, only 2 out of 33 cases had more than one queen (Elst & Gadau, 2018).

Workers on the trails engaged in active interspecific contact, but we did not observe trophallaxis between them. Nevertheless, in a mixed colony, workers readily recognised each other as 'one of their own'. Based on the data obtained, it can be assumed that the existence of mixed colonies for a period of 3 to 6 years is possible if at least one queen of the host species is preserved, which is quite possible with oligogyny.

Another possibility, suggested by Donisthorpe (1915), is that a colony of the host species that has lost its only queen can accept the queen of the temporary social parasite species. This has been confirmed for *L. fuliginosus*, whose queens were adopted by orphaned colonies of *L. umbratus* (Mattheis, 2003). This option is unlikely in our case because the mixed colony contained too many *L. brunneus* workers, which most likely had several queens to maintain such a number. The question of the simultaneous founding of a new colony by one or more queens in *L. umbratus* and *L. fuliginosus* has not been finally resolved; according to some data, this process can be carried out by pleometrosis (Seifert & Buschinger, 2002; Mattheis, 2003).

Conclusions

The hypotheses considered are establishment of a *L. brunneus* colony in 2017 and introduction of a *L. umbratus* queen in 2020, or relocation of a mature *L. brunneus* colony to a new habitat in 2020 and the subsequent invasion of a *L. umbratus* queen. It is assumed that there is more than one queen in a *L. brunneus* colony, which explains its significant size (about 50 thousand workers).

Population calculations for the mixed colony showed a numerical advantage for *L. brunneus* (48,880 individuals) compared with *L. umbratus* (24,433 individuals). Mathematical modelling of growth dynamics confirmed inconsistency of presence of a single *L. brunneus* queen and suggested an alternative explanation for the maintenance of high *L. brunneus* abundance: presence of multiple queens.

Mixed colony hypotheses suggest involvement of several *L. umbratus* and *L. brunneus* queens. Another finding of our study is a change in the lifestyle of *L. umbratus* workers as part of a mixed colony, participating in the movement along the trail and actively interacting not only with workers of their own species but also with *L. brunneus*. The presence of such mixed trails reinforces the mixed scent of the colony and allows both species to coexist for longer. This is also facilitated by antenna contacts when workers of both species recognise each other and do not treat each other with hostility.

In summary, the study provides an overview of development of a mixed colony, including activity, abundance and hypotheses for its formation and evolution.

Development of mixed colonies with a coexistence period of longer than 3 years, during which more than 1 or 2 generations of workers of the host species change, is the most probably possible under the condition of oligogyny in the host species.

This was confirmed by our observations of the same mixed colony in the spring (May) of 2024. Workers of both species were present on the trail.

Similarly, in the summer of 2023 (June), the continued coexistence of a mixed colony N 2 of *L. fuliginosus* and *L. umbratus* in the city of Rivne (Ukraine, see Stukalyuk et al., 2021), which we observed earlier, was also confirmed.

Authors' contributions: S. Stukalyuk — statistical data processing and article writing, Y. Radchenko — mathematical model building and testing, A. Akhmedov —video files processing and video processing algorithm creation, V. Stelia — provision of footage and video filming.

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