

Mixed colonies of *Lasius umbratus* and *Lasius fuliginosus* (Hymenoptera, Formicidae): when superparasitism may potentially develop into coexistence: a case study in Ukraine and Moldova

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Abstract

In 2015, in the territory of Kyiv (Ukraine) and in 2012-2017 in Transnistria (Moldova), two mixed colonies consisting of two species of ants – *Lasius fuliginosus* and *L. umbratus* were observed. Another 2 mixed colonies were discovered in 2021 in the territory of the city of Rivne (Ukraine). The aim of the study was to describe the observed mixed colonies, analyze the interaction of workers of two species on the trails, and by mathematical modelling to determine the probability of preserving queens of both species as part of a mixed colony. On the trails of the colonies from Kyiv and Rivne, the number of workers of *L. fuliginosus* is slightly higher than that of *L. umbratus*, so is the number of *L. fuliginosus* workers in the colony. Workers of both species were making contact both within the same species and interspecific contacts, but trophobiosis with aphids has been recorded only by *L. fuliginosus*. The most likely of the 4 options considered was the survival of the queens of both species, otherwise the dynamics of the population over 6 years of development (total population of the mixed colony from Kyiv: 27 thousand workers of *L. umbratus*, 72 thousand workers - *L. fuliginosus* and two colonies with same population from Rivne) is difficult to explain. The possibility of such mixed colonies co-existing for at least a few years is debated.

Keywords: *Lasius umbratus*, *L. fuliginosus*, mixed colonies, mathematical modelling, trails.

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Introduction

Ants of different species usually inhabit separate nests. However, there are some exceptions. A rudimentary form of symbiosis, plesiobiosis, involves finding nests of different species in close proximity to each other (Czechowski, 2004). The nests themselves exist as separate units and their populations do not mix, i.e. there may be no direct interaction between the species.

Plesiobiosis may be promoted by lack of nesting sites (between *Lasius niger* (Linnaeus, 1758) and *L. flavus* (Fabricius, 1782), *L. platythorax* Seifert, 1991 and *Myrmica rubra* (Linnaeus, 1758)).

A peculiar kind of commensalism was described by Ito *et al.* (2004) in one of the species of the genus *Camponotus* Mayr, 1861 (in the article given as *Camponotus* sp.).

Guided by trace pheromones, *Camponotus* sp. foragers gain access to the food residues of *Crematogaster inflata* Smith, F., 1857, and feed on these residues without causing damage to the "host" species. According to the researchers, the success of *Camponotus* sp. contributes to its similarity with the "host" species in colour (Batesian mimicry). *Crematogaster inflata* has always tried to attack *Camponotus* sp. This suggests that *Camponotus* sp. is a commensal with *C. inflata*, and the similarity of coloration does not help to avoid the attack of *C. inflata*.

Some species use the trails of the "host" species to gain access to its food resources. Such relationships have been described for *Gnamptogenys menadensis* (Mayr, 1877) and *Polyrhachis rufipes* Smith, F., 1858 (Gobin *et al.*, 1998); *Camponotus lateralis* (Olivier, 1792) and *Crematogaster scutellaris* (Olivier, 1792) (Menzel and Blüthgen, 2010), and *C. lateralis* and *Crematogaster schmidti* (Mayr, 1853) (Stukalyuk and Radchenko, 2011). As for *Camponotus lateralis* and *Crematogaster scutellaris*, *Crematogaster* workers always attack *Camponotus lateralis* workers, but are less aggressive towards workers using the same trail as *Crematogaster* (Menzel and Blüthgen, 2010). *C. lateralis* and *Crematogaster schmidti* also shows similar behaviors- *Crematogaster* attacks them, while *C. lateralis* avoids aggression (Stukalyuk and Radchenko, 2011). Another variant of coexistence of ants within the same nest is social parasitism.

The phenomenon of parasitism is quite widely and variously represented among ants: from temporary social parasitism, characteristic of red wood ants, as well as some representatives of the genus *Lasius*, to real social parasitism ("slavery" - in *Polyergus rufescens* (Latreille, 1798), *Strongylognathus* spp.). Some ants are obligate kleptoparasites (*Solenopsis fugax* (Latreille, 1798)) (Radchenko, 2016; Seifert, 2018).

Temporary social parasites establish their colonies at the expense of the colony of the "host" species, when their females enter the nests and kill the local queen. These include *Lasius umbratus* (Nylander, 1846), a parasite of *Lasius niger*, and the superparasite species *Lasius fuliginosus* (Latreille, 1798), whose colonies appear and grow, in turn, mainly at the expense of the parasite *L.*

umbratus. *L. fuliginosus* is the only known superparasite species for Europe (Seifert, 2018). B. Seifert (2018) described cases of finding such mixed colonies consisting of *L. fuliginosus* and a second species: *L. umbratus* (7 colonies), *L. sabularum* (Bondroit, 1918) (2), *L. jensi* Seifert, 1982 (2), *L. meridionalis* (Bondroit, 1920) (1) and *L. bicornis* (Foerster, 1850) (1). The number of species on which *L. fuliginosus* parasitizes is quite large, even in laboratory conditions the development of colonies of this species is possible due to *L. niger*.

Both species (*L. fuliginosus* and *L. umbratus*) are characterized by similar and diametrically opposite features of biology: a) large colony size (tens to hundreds of thousands of workers); b) construction of cardboard nests (mainly at the base of trees); c) different tiers of habitat and foraging, *L. umbratus* - a geobiont that rarely appears on the surface of the soil, *L. fuliginosus* - a dendrobiont that has trails on the surface of the soil and on tree trunks; d) both species use the odorous secretion of the mandibular glands in defense or attack (citronellol in *L. umbratus* (Blum *et al.*, 1968), and dendrolasine in *L. fuliginosus* (Bernardi *et al.*, 1967)).

The phenomenon of temporary parasitism in these two species in some cases, as our observations have shown, can develop into co-existence. One of the authors (SS) in 2015 discovered a mixed colony of *L. umbratus* and *L. fuliginosus* on the territory of Kyiv (Ukraine) and recorded workers of both species on the ground, on the trail and in the territory of the forage area. One of the first observations was made back in 1897 by the British scientist Donisthorpe (1915), who observed a mixed colony of two species and their workers entering and exiting the same passages. This phenomenon was described in more detail by the Dutch scientist Lodeizen (1946), who observed such a mixed colony for three years (1939-1941). Subsequently, the mixed colonies *L. umbratus* and *L. fuliginosus* were observed by the Polish myrmecologists B. Pisarski, and, later, W. Czechowski (W. Czechowski, personal communication). Thus, the very existence of mixed colonies of *L. umbratus* and *L. fuliginosus* is possible, although it is observed in isolated cases. Given that *L. fuliginosus* is a parasite of *L. umbratus*, the existence of such mixed colonies is a natural phenomenon. However, this existence

of two species in the same nest is short-lived – after the murder of the *L. umbratus* queen, only one generation of workers of this species, possibly another generation of them, hatched from eggs laid before the murder of the *L. umbratus* queen.

In favor of the option of a longer coexistence of mixed colonies of two species, with the preservation of their queens, we give several arguments.

1. The presence of two species of workers on the trails passing on the surface. We have observed hundreds of *L. fuliginosus* colonies, but no *L. umbratus* workers have ever been identified on their trails, for which access to the surface is excluded during all periods of the life cycle, except during the mating summer.

2. Workers of both species in the nest. The penetration of the queens of *L. fuliginosus* is unlikely in the colonies of *L. umbratus*, numbering tens to hundreds of thousands of workers, since it becomes almost impossible to find the queen of the host species and destroy it. In addition, according to some data, oligogyny is possible in *L. umbratus* (Seifert, 2018), so after the murder of one queen of this species, another may well persist.

3. If the population of both species in the colony is large and numbers tens of thousands – hundreds of thousands, in order to reach the number of workers comparable to *L. umbratus*, the queen (or queens) of *L. fuliginosus* will need more than one year to increase to such a population. The presence of observations of workers of two species in the same colony for 2 years or more (i.e. longer than the life cycle of workers of one generation) will allow us to confidently assert the fact of the preservation of queens of both species within the same colony.

All these assumptions, as well as the data of other researchers, allowed us to assume that the existence of mixed colonies of the two species is possible. This article consists of three parts - biological, which describes the features of the biology of mixed colonies that we have established; mathematical, which analyzes the models of population growth of two species within one mixed colony, and chemical, which analyzes the literature data on pheromones (trace and alarm) used by the two species. According to the authors of this article, trace pheromones played a crucial role in the possibility of co-existence of these two

species when their workers moved together on the trails. Therefore, it is necessary to analyze the chemical structure and proximity of the trace pheromones of *L. umbratus* and *L. fuliginosus* by chemical nomenclature, and compare them with those of other ant species of the genus *Lasius* Fabricius, 1804.

The aim of this study is to describe the case of two mixed colonies discovered by the authors: *L. umbratus* and *L. fuliginosus*. The tasks set by the authors included: a) to study the characteristics of the activity of workers on the trail and forage; b) to identify whether both species live together, in the same nest, or coexist in two different nests (plesiobiosis); c) to analyze the literature data on the chemical composition of trace pheromones in *L. umbratus* and *L. fuliginosus* and other ant species for the similarity of their structure.

Materials and Methods

Nest details

Observations were made on a single mixed colony, *L. umbratus* and *L. fuliginosus*, containing workers of both species on trails and inside the nest. The colony was discovered in the territory of Kyiv in July, 2015. The place of discovery is Zhukov Island (50°21.768 N, 30°33.731 E), located on the southern outskirts of the city. Meadow communities predominate, as well as floodplain forests, often with a high level of shading formed by *Acer negundo* undergrowth. The first tier consists of trees of oak petiolate (*Quercus robur*), black poplar (*Populus nigra*), and white poplar (*Populus alba*), the second tier consists of elm trees (*Ulmus laevis*) and maple undergrowth (*Acer negundo*). The colony lived in the trunk of an old oak tree (1m in diameter, Fig. 1A), the nest and its exits were located under the base of the trunk (Fig. 1B). Along the trunk a forage trail of both species were observed. The neighboring trees were not occupied by these ants. The oak grows on the outskirts of the clearing, at the entrance from the dam to the territory of Zhukov Island, in a recreation zone.

On the second visit in May 2018, the colony could not be found. The 4 colonies of *L. fuliginosus* that are located nearby and the distance to them are taken into account. The data collected in 2015 were used in processing of the obtained material.



A



B

Figure 1. A: *Quercus robur* tree, populated by ants of mixed colony in Kyiv; B: nest entrance (marked by arrow).

The second colony was located in the vicinity of Rybnitsa (coordinates 47°65.0982 N, 29°09.8723 E, Moldova, Transnistria), on the territory of a dacha plot. In this colony, the population of *L. fuliginosus* workers was smaller, and the trail was mostly underground. The length of the trail (which was established by excavation) was 26m. The mixed colony was observed for 5 years, from 2012 to 2017, i.e. during this period, at least 4 generations of workers of both species should have changed in it. Unfortunately, we did not take into account the total population of the colony, but according to approximate estimates, there are about 30 thousand *L. umbratus* workers and about 40 thousand of *L. fuliginosus* workers.

In July 2021, in the territory of the city of Rivne (Ukraine), observations were carried out over two more mixed colonies. The first of the colonies (coordinates 50°61.37522 N,

26°30.27270 E) had one trail, and ants from it visited 1 birch tree (*Betula pendula*), at the base of which their nest was located (trunk diameter 0.48m). The second colony was larger, and ants from it controlled 3 birches (trunk diameter 0.6–0.7m), at the base of two of which there were nest entrances (colony coordinates 50°60.9631 N, 26°30.8069 E). There were 2 trails between the trees.

Trails were also found on the trunk of each of the trees in both colonies. Both colonies, as observed by one of the authors (AS) of the article, were at least 4 years old.

Research methods

Antennal contacts on the trail: A video file with ants of both species moving along the trail, lasting 32 seconds, was processed for further statistical analysis. A video record was made (for a period of 2 seconds) of the contacts of all ants that moved along the trail:

the number of antennal contacts among workers of same species as well as between two different species. To do this, we studied the trajectory of each of the ants moving along the trail, the number of contacts with individuals of their own species, with individuals of another species. Ants that moved on trails from aphid feeding grounds were taken into account - they had a swollen abdomen. This is important because it is possible to assume the role of workers of each species on the trail leading to the aphid colonies. In general, the analysis of the file takes into account the trajectories of movement of 16 *L. umbratus* workers and 21 *L. fuliginosus* workers. The total number of 2-second counts was 117 for *L. umbratus*, 99 for *L. fuliginosus*.

Similar calculations were made for two colonies from the city of Rivne. For this, in each of the two colonies, 4 videos were filmed, 3 minutes long, for trails on the ground, and the same number for the trails on tree trunks. For the trails passing on the ground, the trajectories of 135 *L. fuliginosus* workers and 18 *L. umbratus* workers were taken into account. 855 counts per 2 seconds for *L. fuliginosus*, 225 counts for *L. umbratus* were performed. For the trails passing along tree trunks, the trajectories of 57 *L. fuliginosus* workers (405 counts) and 10 *L. umbratus* (64 counts) workers were taken into account, respectively.

Three groups of parameters were designated, among which statistical comparisons were made: a) antennal contact (*umbratus-umbratus*, *umbratus-fuliginosus*, *fuliginosus-fuliginosus*); b) the total number of ants of each species that were in the field of view for 2 seconds of recording; c) the number of ants of each species that did not participate in antenna contact.

Activity on the trails: For two colonies from the city of Rivne, we carried out detailed surveys of workers' activity. This is necessary to establish the ratio of the number of workers of both species of ants on the trails. In addition, on the basis of these data, it becomes possible to calculate the number of foragers in the colony, as well as the total population of the colony.

The counts were carried out at 7.00 PM local time, when the ants were most active, after the air temperature dropped to 26-27°C. Each count of activity was carried out

for two minutes. All ants of both species passing under a gate-shaped barrier above the trail were counted. They did not put a gate on the tree, but chose a place on the trunk, along which it was easy to navigate (a branch, a crack in the bark). For both colonies, 30 counts each were made for a trail on the tree and for a trail on the ground.

Foraging activity: To check the foraging activity of both ant species, baits were laid out at an equal distance (0.5 m) along the trail on the ground in each of the two colonies. Preliminarily, killed individuals of the Turkmen cockroach (*Shelfordella tartara*) were used as bait. In total, 60 baits were laid out, 30 in each colony. The counts of the number of ants of both species were kept until the bait (1 cockroach = 1 bait) was moved to the nest. For colony 1, this time was 1 hour (6 counts of each bait, 180 counts in total), for colony 2 - 30 minutes (3 counts of each bait, 90 counts in total).

Protection of the nest: To check the participation of both species in the protection of the nest, in colony 2, 100 pre-collected workers of *Lasius niger* were planted to the nest entrance. A video was filmed (duration 12 minutes), during the processing of which the number of *L. fuliginosus*, *L. umbratus* ants that came out to defend the nest, the number of their attacks against *L. niger* workers for each 1 minute of video was recorded.

Excavation of the nest: For both colonies from the city of Rivne, excavations were carried out in order to take into account the approximate ratio of ants of both species inside the nest. Excavations were carried out to the level of the cardboard part of the nest.

Calculations of the number of foragers and the total worker population in the mixed colony: The number of foragers, as well as the total number of workers of the two species in the nest, was established using the formula proposed by Zakharov (1978, 2015). Foragers make up an average of 13% of the colony population, so it is possible to calculate the colony population for each of the species.

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

A- the number of foragers (in hundreds of individuals); I- the number of workers (individuals per 1 minute on the trail). The accuracy of the method is $P = \pm 1.6\%$.

To calculate the number of workers, 3 observations of the trail for 5 minutes were sufficient, while taking into account the number of workers passing in one direction along the trail. Formula 1b was used for calculation of the total population of the colony:

$$N = 7.7A \quad (1b)$$

Number of foragers on average should be equal to 12.98% (13%) of the total number of workers in the colony (Zakharov, 2015). These data agree with others, according to which the number of foragers in *L. fuliginosus* may be 15% of the total population (Hennaut-Riche *et al.*, 1980)

Mathematical modeling

In order to create a mathematical model, we have made assumptions that are based on the literature data.

1. One queen could support a population of up to 60 thousand workers at a time. These data are given for *L. niger* (Boomsma *et al.*, 1982), however, we did not find data for *L. umbratus*, *L. fuliginosus*. Taking into account the fact that all species have physogastric queens, but the colony population of *L. umbratus*, *L. fuliginosus* is on average 2-10 times larger (on average, 13 thousand in *L. niger*, 20-30 - *L. umbratus*, 100 thousand - 1 million in *L. fuliginosus* (Radchenko, 2016) or even up to 5 million (Zakharov, 2015)), we assume that the queens of these species are more fertile, and, consequently, faster rates of colony growth in *L. umbratus*, *L. fuliginosus* compared to *L. niger*. By knowing the total population of a colony, we can guess how many queens of each species are there, provided the population that each queen is able to support is already known. For *L. umbratus*, we leave the figure at 60 thousand, because on average, its colonies are slightly larger than those of *L. niger*, for *L. fuliginosus*, we allow a figure of 100 thousand and the growth rate is 5 times higher compared to *L. umbratus*. *L. umbratus*, in turn, develops 5 times more intensively than *L. niger*, since its queen (as well as the queen of *L. fuliginosus*), begins oviposition not at the expense of its own reserves of the fat body, but at the expense of a full-fledged abundant nutrition in the already formed colony of the host species.

2. The population in a mixed colony (calculated according to the Zakharov

formula) was 72.816 workers of *L. fuliginosus* (of which 9.466 are foragers) and 27.740 workers of *L. umbratus* (3.606 foragers). For colony 1 from Rivne, the population was 53.651 workers of *L. fuliginosus* (6.967 foragers) and 24.261 workers of *L. umbratus* (3.151 foragers). For colony 2 from Rivne, the population was 65.043 workers of *L. fuliginosus* (8.447 foragers) and 22.607 workers of *L. umbratus* (2.935 foragers).

3. At the time of the penetration of the queen of *L. fuliginosus* into the colony of *L. umbratus*, there were about 5 thousand workers. We believe that, invasion of small colonies of *L. umbratus* is unlikely, since the mass oviposition of *L. fuliginosus* requires an abundant supply of food, and consequently, a large number of foragers of *L. umbratus* collecting it. Therefore, we chose the number of *L. umbratus* workers corresponding to the average number of workers in the colony.

4. The period of egg laying for two species is from spring (end of March) to August inclusive, by analogy with *L. niger* (Kipyatkov, 2007).

5. The life expectancy of the queen in both species is from 5 years to 20 years and the worker - one season. Workers come out of the cocoons in June and can live until the next July-August. Therefore, there is a change of generations, and each subsequent one should exceed the number of the previous one. The exact life span of a worker is taken by analogy with the data for *L. niger*, whose workers on average live a little more than a year – 434 days in young colonies and 309 days in an adult colony (Kramer *et al.*, 2016). The maximum life expectancy of workers was 1094-1129 days, but less than 1% of workers live to this age. The majority of workers (95 %) die out the very next year after birth.

6. From eggs laid in the summer, a generation of workers comes out in the fall, if the foundation occurred in June, if later - then in May-June of the following year. It is only one generation of workers per year (Seifert, 2018).

7. The rate of population growth is the same for the two species, but different depending on the age of the colony. In the laboratory colonies of *L. niger*, it is 300, 1500 and 5000 workers for one, two and three years after the foundation of the colony (Buschinger, 2016). Further growth we built on this trend, taking into account the specific feature (greater

fecundity) of *L. umbratus* and *L. fuliginosus*. In addition, we used data on the growth rate of honeybee colonies (*Apis mellifera* L.), which increased their number from 6 thousand workers to 15 thousand in 120 days (Rangel *et al.*, 2013).

8. From the moment of the penetration and murder of the queen of *L. umbratus* (or a failed murder attempt, and preservation of the queens of both species), the oviposition of *L. fuliginosus* begins only after 5-14 days (Seifert, 2018). Penetration occurs after the mating summer, that is, from the beginning of June-July.

When modeling the behavior (development) of living systems, the question arises about the method by which this could be achieved. Fortunately, despite the diversity of living systems, they have specific features. This allows us to develop common approaches for the construction of such models. As a rule, models take into account the following:

- biological systems are complex multicomponent, spatially structured, the elements of which have individuality;
- these are multiplying systems (capable of autoreproduction);
- they are open systems that constantly pass through the flow of matter and energy;
- biological objects have a complex multi-level system of regulation.

Conditionally, all mathematical models of biological systems can be divided into regression, qualitative, and simulation models.

Currently, due to the development of computing and graphics capabilities of computers, simulation models play an increasingly important role.

The essence of simulation modeling is to study a complex mathematical model using computational experiments and processing the results of these experiments.

The process of building a simulation model is as follows. In a formalized form accessible to the computer (in the form of equations, graphs, logical relations), everything known about the system is set. Then the options are played on the computer for what the totality of this knowledge can give for certain values of the external and internal parameters of the system.

Simulation models of specific living systems take into account the available information about the object as much as

possible. When creating a simulation model, you can afford a high degree of detail when selecting the variables and parameters of the model. At the same time, the model may be different for different authors, since there are no exact formal rules for its construction. The results of machine experiments depend not only on the relations inherent in the model, but also on the organization of the complex programs implementing the model, and on the mechanism of conducting machine experiments (Riznichenko, 2002).

In our case, the main tasks of simulation modeling were:

- * testing the hypothesis about the interaction of individual ant species;
- * forecast of their behavior when changing internal characteristics (terms of invasion, its conditions);

When constructing a simulation model of the process of coexistence of colonies of *L. fuliginosus* and *L. umbratus*, it is considered that the population size changes over time (grow, fall, possibly fluctuate) due to the changes in the productivity of the queen of the corresponding species.

In our case, this is a systemic factor that limits the growth of the population.

In the classical models of mathematical ecology, this phenomenon is described by the equation of logistic growth (Riznichenko, 2002):

$$\frac{\partial x}{\partial \tau} = r \cdot x \cdot \left(1 - \frac{x}{K}\right) \quad (2)$$

x – colony size;

τ – time;

r – growth rate constant;

K is the population capacity.

This equation has an analytical solution:

$$X(\tau) = \frac{X_0 \cdot K \cdot e^{r\tau}}{K - X_0 + X_0 \cdot e^{r\tau}} \quad (3)$$

X_0 – initial number of specimens.

This solution demonstrates two features of growth: at small values of X , the number increases exponentially, at large values, it approaches a certain limit of K .

Formula (3) describes the kinetic curve, that is, the dependence of the population size on time. In contrast to (3), assuming that the number of eggs $Y(x)$ that the queen lays does not depend on time, but on the number of worker ants of a given species, we

obtained an expression for calculating her productivity over a weekly cycle:

$$Y(x) = \frac{Y_0 \cdot K \cdot e^{rx}}{K - Y_0 + Y_0 \cdot e^{rx}} \quad (4)$$

Y_0 – initial productivity.

The survival rate of the pupae is also determined by the number of workers of this species. The total population of the anthill of a species has been calculated taking into account the average life expectancy of a working ant equal to 400 days, given a mortality percentage equal to 10% of the number of overwintered ones.

As part of our work, we considered the following options, or variants of development:

1 – 1 queen of *L. umbratus* was killed and 1 queen of *L. fuliginosus* settled;

2 – both queens are alive after the introduction of 1 queen of *L. fuliginosus*;

3 – 1 of the 2 *L. umbratus* queen is alive, 2 queens of *L. fuliginosus* have been introduced;

4 – two queens of *L. umbratus* are alive after the introduction of 2 *L. fuliginosus* queens.

Only 1 out of these 4 options provide for the presence of two queens in both species. Only two colonies of *L. fuliginosus* out of 33 surveyed in Germany had more than one queen (Elst and Gadau, 2018). According to other data, *L. fuliginosus* might have several queens (Czechowski *et al.*, 2012). *L. umbratus* is mainly characterized by a single queen, but oligogyny is also possible (Seifert, 2018). All this serves as a basis for considering not only options with monogyny, but also with oligogyny.

Statistical analysis

Statistical calculations were performed with the Past v. 4.03 software. The data were checked for the normality of the distribution, in case of non-compliance with the normal distribution, nonparametric analysis methods were used. Differences between groups (the number of antennal contacts, between one species and both species; the average number of ants of each species for 2 seconds of accounting; the number of ants of both species that did not participate in antennal contact) were checked using the Kruskal-Wallis test (K-W) for equal medians. In the case of significant differences, the Mann-Whitney test (M-W, with Bonferroni corrected p value) was used. The average values of the parameters are

shown in the form of Barchart-boxplot (Past v. 4.03), the mode of violin and box.

Results and Discussion

Aft section: For the mixed colony in Kyiv, it includes only one tree and one trail, which is less than the average for *L. fuliginosus* from the same area, in the territory of mixed forests (Stukalyuk, 2017). On an average, there are 3.4 ± 0.2 trails per single-species colony of *L. fuliginosus*, 13.0 ± 2.3 trees visited by ants. The activity of ants on the trails averages 50.7 ± 6.5 workers per 2 min for 2015, with maximum values up to 150-200 workers per 2 min. For a mixed colony in Kyiv, this indicator was 87.0 ± 9.6 workers of *L. fuliginosus* and 19.6 ± 4.8 workers of *L. umbratus* for 2 minutes of accounting. For colony 1 from the city of Rivne, these parameters were 64.9 ± 1.4 *L. fuliginosus* and 5.9 ± 0.42 *L. umbratus* workers; for colony 2: 72.0 ± 3.1 *L. fuliginosus* and 9.2 ± 0.7 *L. umbratus* workers, respectively. Thus, the level of the activity of *L. fuliginosus* workers on the trails in a mixed colony is different from single-species ones. The workers of *L. umbratus* on the trails were more than 5.0 times less numerous than the workers of *L. fuliginosus* (at $p < 0.05$). The expansion (and later, possibly, relocation) of the colony could be prevented by the open spaces between neighboring trees, which are trampled earth.

The death of mixed colonies in Kyiv and in Transnistria: A second inspection in 2018 showed that the colony died, possibly more than a year ago. The oak was occupied and visited by other ant species - *Lasius brunneus* (Latreille, 1798), *Camponotus fallax* (Nylander, 1856), *Dolichoderus quadripunctatus* (Linnaeus, 1771). The excavations revealed the abandoned remains of cardboard structures, but without remains of the bodies of the dead ants.

The nearest colonies of *Lasius fuliginosus* were located at distances of 300-700m, the closest at 150m, separated by a road and had a size of an order of larger magnitude. Most likely, a mixed colony of both species, for some reason, died. Nearby solitary oaks were occupied by colonies of *Lasius brunneus*, sometimes *Camponotus fallax*, *Dolichoderus quadripunctatus*. The trees of other species were dominated by *Lasius niger*.

Mixed colonies of *L. umbratus* & *L. fuliginosus*: when superparasitism may develop into coexistence

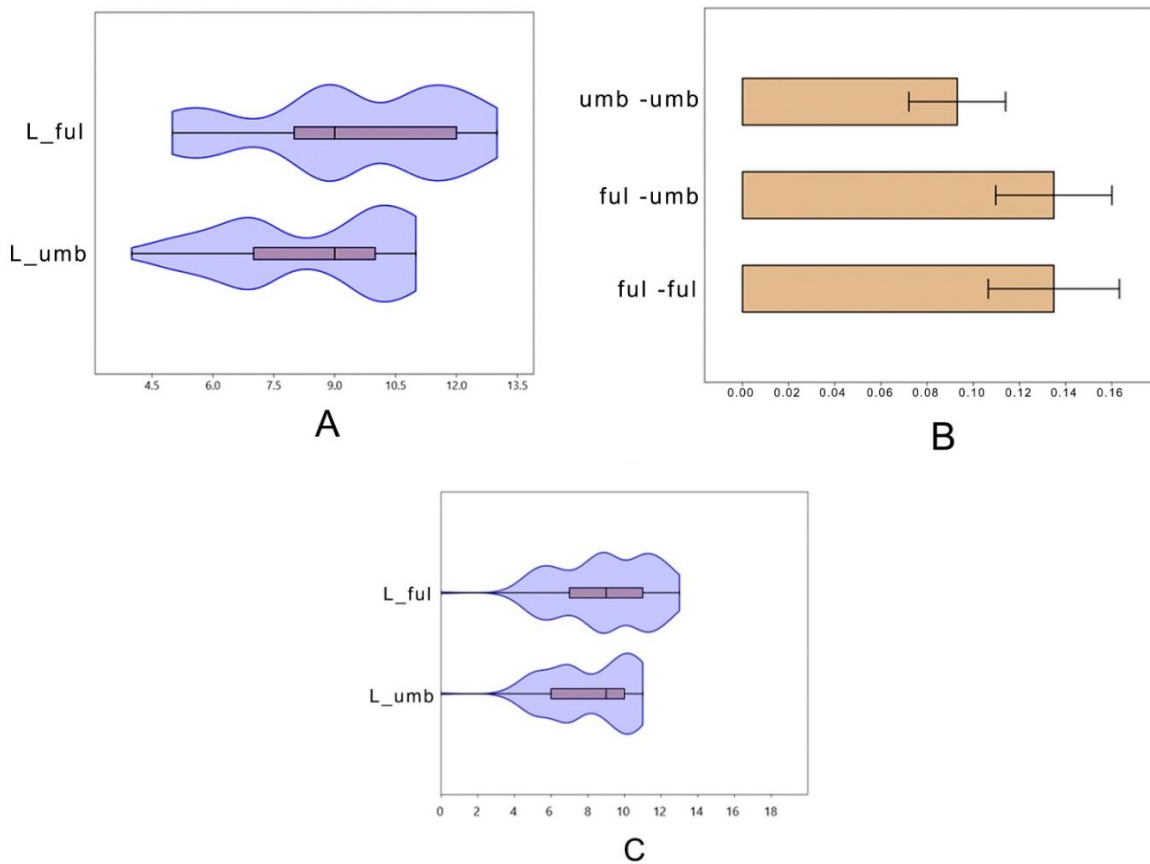


Figure 2. **A:** The average number of workers of *Lasius umbratus*, *L. fuliginosus* for 2 seconds of accounting on the trail of a mixed colony (Kyiv); **B:** The average number of workers of *Lasius umbratus*, *L. fuliginosus* for 2 seconds of accounting, who participated in antennal contact on the trail of a mixed colony (Kyiv); **C:** The average number of workers of *Lasius umbratus*, *L. fuliginosus* for 2 seconds of accounting, who did not participate in antennal contact on the trail of a mixed colony (Kyiv).

The mixed colony from Transnistria also died, both species disappeared. This was consistent with the data for Kyiv and indicates that if both queens are preserved, the colony will most likely don't last longer than 5-7 years.

A mixed colony may break up due to the death of one of the queens. The colony we studied could have appeared under the following circumstances: when the queen of *L. fuliginosus* entered, the queen of *L. umbratus* was not killed or there might have been several queens in the colony of *L. umbratus*.

Antennal contacts on the trail

Kyiv colony: It was found that on the trail, the average number of workers of both species differed in two seconds of accounting - there were more *L. fuliginosus* workers (K-W: $p=9,179 \text{ E-}06$) (Fig. 2A). Thus, on the trail, the number of *L. fuliginosus* workers prevails. It may be due to the peculiarities of the

biology of *L. umbratus* - in contrast to *L. fuliginosus*, foraging by *L. umbratus* is carried out underground, not on the surface. The very fact of finding workers of *L. umbratus* on the mixed-colony trail is a very unusual feature of their biology.

There is a positive relationship between the number of workers of both species on the mixed-colony trail (M-W, $p=9.195 \text{ E-}06$). Workers of both species made antennal contacts, both intraspecific and interspecific (Fig. 2B). At the same time, no significant differences were found between the number of contacts in 2 seconds (K-W: $p=0.4513$). That is, workers of both species were equally often in contact with individuals of their own species, as well as with individuals of another species.

Significant differences were found between the number of workers of both species who did not participate in antennal contact, but simply moved along the trail of the mixed colony (K-W: $p=0.0004891$) - the

number of *L. fuliginosus* workers is greater, while the differences are significant (M-W: $p = 0.0004898$) (Fig. 2C).

It follows from this that the number of *L. fuliginosus* workers on the trail is slightly higher than that for *L. umbratus*, and therefore the number of *L. fuliginosus* workers that do not participate in antennal contact is also higher. It is obvious that the duration of the video is too short, so we received updated data on the number of workers of both species after the trail surveys. At the same time, workers of both species are willing to contact both within the same species and interspecific contacts. Such behavior on the trails in a mixed colony of two parasitic species is possible only in the case of a close structure of pheromones-traces, alarm pheromones, etc., or in the case of an exchange of cuticular hydrocarbons.

Rivne colony: Significant differences were established for the number of antennal contacts between workers of two species on a trail passing along the ground (K-W: $p = 9.926E-12$, Fig. 3A). The maximum number of antennal contacts were between workers of *L. fuliginosus*; interspecific contacts are somewhat less intense (M-W: $p = 0.02448$). Contacts between workers of *L. umbratus* are the least frequent, less frequent than interspecific contacts (M-W: $p = 1.864E-06$), and contacts between workers *L. fuliginosus* (M-W: $p = 5.357E-10$).

On the trails passing along the trunk of trees, no antennal contacts between workers of *L. umbratus* were recorded. Differences between the other two categories of contacts are significant (K-W: $p = 0.02547$, Fig. 3B). The number of contacts between workers *L. fuliginosus* is, on an average, greater than the number of interspecific contacts (M-W: $p = 0.02561$). The lower number of *L. umbratus* workers on the trails passing on the ground, and their almost complete absence on the trails passing through tree trunks, is the reason for the low number of intraspecific contacts compared to other categories.

Thus, in a mixed colony, there is a full-fledged coexistence of the workers of the two species, with antennal contacts, following the trails (possibly by the trace pheromones of *L. fuliginosus*). The very fact of the presence of *L. umbratus* workers on the trails outside the nest indicates a change in their lifestyle. They are less visually oriented on the surface, and their eyes contain up to 60 ommatidia

(Blum *et al.*, 1968). Therefore, it can be assumed that the trace pheromones of *L. fuliginosus* for *L. umbratus* will play a key role when moving outside the nest.

Activity on trails

For colony 1 from the city of Rivne, a significant difference was found in the number of ants on the trails passing along the surface of the earth (K-W, $p = 2.019E-20$, Fig. 4A). There are more *L. fuliginosus* workers on the trails than *L. umbratus* workers (M-W, $p = 7.44E-11$, Fig. 4A). In colony 2, the same pattern is observed (M-W, $p = 1.76E-10$). There is no statistically significant difference between the number of *L. fuliginosus* workers on the trails in the two colonies (M-W, $p = 0.4476$). The number of *L. umbratus* workers on the trails is higher in colony 2 than in colony 1 (M-W, $p = 0.002647$, Fig. 4A).

The number of ants on the trails passing along tree trunks also differs (K-W, $p = 3.46E-22$, Fig. 4B). The number of workers of *L. fuliginosus* in colony 1 significantly exceeds the number of workers of *L. umbratus* (M-W, $p = 8.49E-11$, Fig. 4B). In colony 2, the situation is similar (M-W, $p = 1.62E-10$). The number of *L. fuliginosus* workers on the tree trunk trail in colony 2 is slightly higher than in colony 1 (M-W, $p = 1.82E-07$). Similarly, the number of *L. umbratus* ants in colony 2 on the trails passing on the trees is higher than in colony 1 (M-W, $p = 2.03E-05$, Fig. 4B).

Foraging activity

The general tendency for both colonies from the city of Rivne is an increase in the number of *L. fuliginosus* and *L. umbratus* on baits over time (Figs. 4C, D). Moreover, the difference in their number is significant between species (K-W, colony 1, $p = 3.22E-26$; colony 2, $p = 2.28E-50$). An increase in the number of *L. fuliginosus* foragers on baits in colony 1 occurs in the first 30 minutes; in the next 20 minutes, their number slightly decreases and grows again after 1 hour (Table 1). For *L. umbratus*, the number of workers in the first 40 minutes increased 10 times (Fig. 4C), then decreased slightly (50 minutes) and again stabilized at the same level, 60 minutes after laying out the baits. The number of *L. fuliginosus* workers on baits did not increase so significantly, by 4 times (Fig. 4C). For colony 2, the number of workers in *L. umbratus* doubled only after half an hour, while the number of workers in *L. fuliginosus* increased by only half (Fig. 4D).

Mixed colonies of *L. umbratus* & *L. fuliginosus*: when superparasitism may develop into coexistence

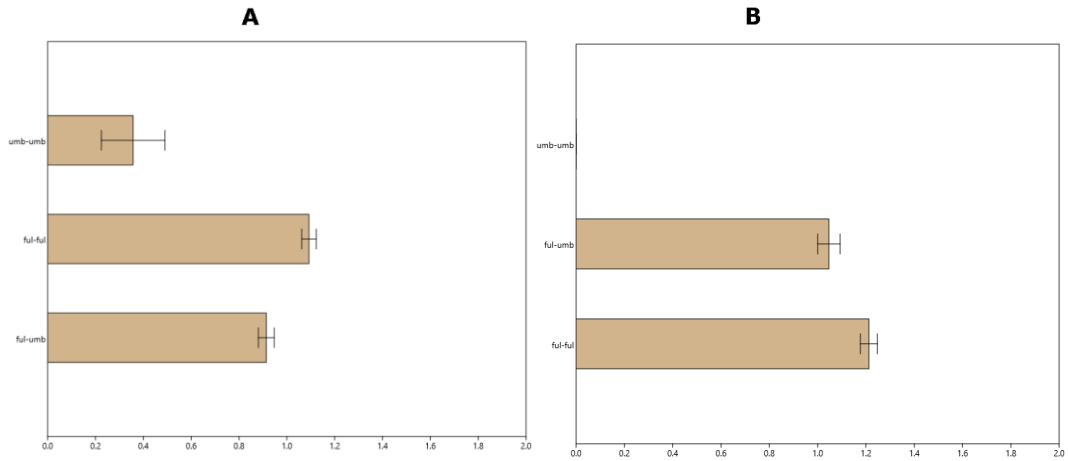


Figure 3. A: The average number of workers of *Lasius umbratus*, *L. fuliginosus* for 2 seconds of accounting, who participate in antennal contact on the trail on the ground of a mixed colony (Rivne); **B:** same, but trail on tree trunk.

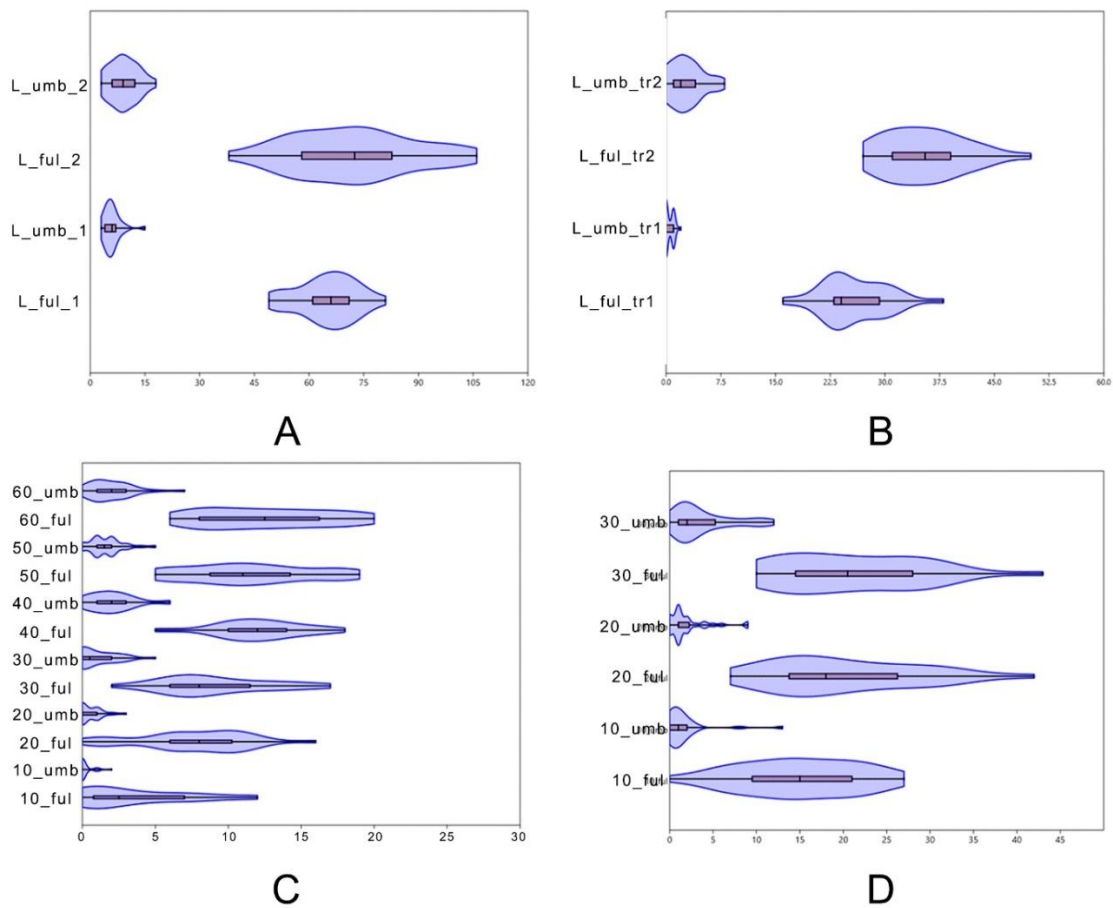


Figure 4. A: Average number of workers *L. fuliginosus*, *L. umbratus* for 2 minutes of counting on the trails passing on the ground in colony 1 (L_ful_1, L_umb_1) and in colony 2 (L_ful_2, L_umb_2), Rivne, Ukraine; **B:** Average number of workers *L. fuliginosus*, *L. umbratus* for 2 minutes of counting on the trails passing on the trunks of trees in colony 1 (L_ful_tr1, L_umb_tr1) and in colony 2 (L_ful_tr2, L_umb_tr2), Rivne, Ukraine; **C:** Average number of workers *L. fuliginosus*, *L. umbratus* attracted on bait in colony 1 during 10 (10_ful, 10_umb), 20 (20_ful, 20_umb), 30 (30_ful, 30_umb), 40 (40_ful, 40_umb), 50 (50_ful, 50_umb), 60 (60_ful, 60_umb) minutes of accounting, Rivne, Ukraine; **D:** Average number of workers *L. fuliginosus*, *L. umbratus* attracted to bait in colony 2 during 10 (10_ful, 10_umb), 20 (20_ful, 20_umb), 30 (30_ful, 30_umb) minutes of counting., Rivne, Ukraine.



A



B

Figure 5. A: *L. fuliginosus*, *L. umbratus* workers on baits, colony 1; B: colony 2, Rivne, Ukraine.

The intensity of foraging in colony 2 also increased with time; there are significant differences between values of 10-30 minutes for both ant species (Table 2). In both species, the number of workers mobilized for bait, increased over time.

It is worth noting that the workers of *L. umbratus* generally did not so much participate in the transport of the bait, but rather moved alongside it. We noted isolated cases of feeding *L. umbratus* workers on bait in both colonies (Figs. 5A, B). From this, it can be concluded that in the outer territory of the forage area, *L. umbratus* workers generally

simply moves along trails, and does not participate in transporting prey, hunting it, or visiting aphid colonies (the latter according to data obtained for the colony from Kyiv).

Protection of the nest

For workers of all three species, significant differences were found in changes in numbers over time (K-W: $p = 4.339E-07$). As the number of *L. fuliginosus* workers increased, more *L. umbratus* workers were mobilized (M-W: $p = 0.0005339$), and the number of *L. niger* workers fell steadily (M-W: $p = 0.002625$). During the first 2 minutes, the number of *L. niger* workers decreased by 5 times; most of them left the landing site immediately after attacks from *L. fuliginosus* (Fig. 6A). After 7 minutes, all *L. niger* workers either disappeared or were killed. The attacks were mainly initiated by the workers of *L. fuliginosus*, but the workers of *L. umbratus* also attacked the enemy (*L. niger*), albeit at a lower frequency (Fig. 6B). With an increase in the number of *L. fuliginosus* workers, the number of their attacks against *L. niger* increased (MW, $p = 0.006477$), increasing number of attacks by *L. umbratus* against *L. niger* (MW: $p = 0.0003906$) and counter attack by *L. niger* against *L. fuliginosus* and *L. umbratus* (MW: $p = 0.0003351$) were also observed. The increase in the number of *L. umbratus* also contributed to an increase in the frequency of their attacks against *L. niger* (MW: $p = 0.02209$) and response attacks of *L. niger* against *L. umbratus* (MW: $p = 0.01589$). It is interesting to note that the number of *L. fuliginosus* workers, as well as *L. umbratus* workers, remained consistently high almost until the end of the video recording, having only slight decrease in the last 2 minutes. This retention of a high concentration of workers of both species can be interpreted.

Excavation of nests

Excavations of the nests of colonies 1 and 2 (Rivne) showed different results. In colony 1, it was possible to only partially open the layer of wood under which the nest was located. Visually, the ratio of workers of the two species was in favor of *L. fuliginosus*, of which there were about 3 times more (we counted 127 workers of *L. fuliginosus* and 43 workers of *L. umbratus*). In colony 2, we managed to excavate the nest down to the cardboard part.

Mixed colonies of *L. umbratus* & *L. fuliginosus*: when superparasitism may develop into coexistence

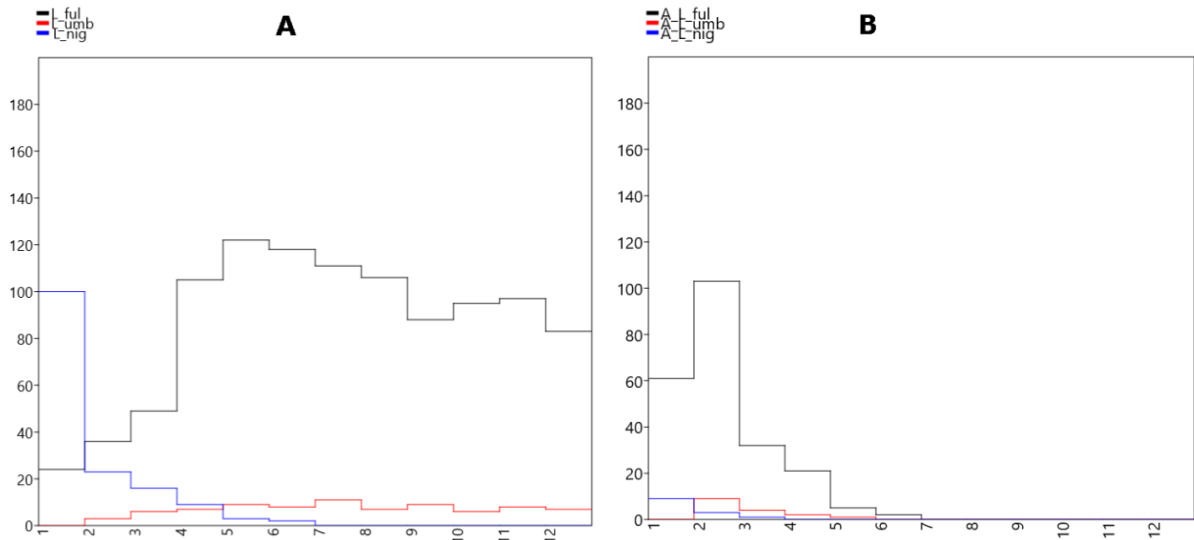


Figure 6. **A:** Number of workers *L. niger*, *L. fuliginosus*, *L. umbratus* / 1 min at the nest entrance after the appearance of 100 workers *L. niger*; **B:** number of interspecies attacks / 1 min. Vertically - the number of workers (or attacks) / 1 minute; horizontally - time, minutes.

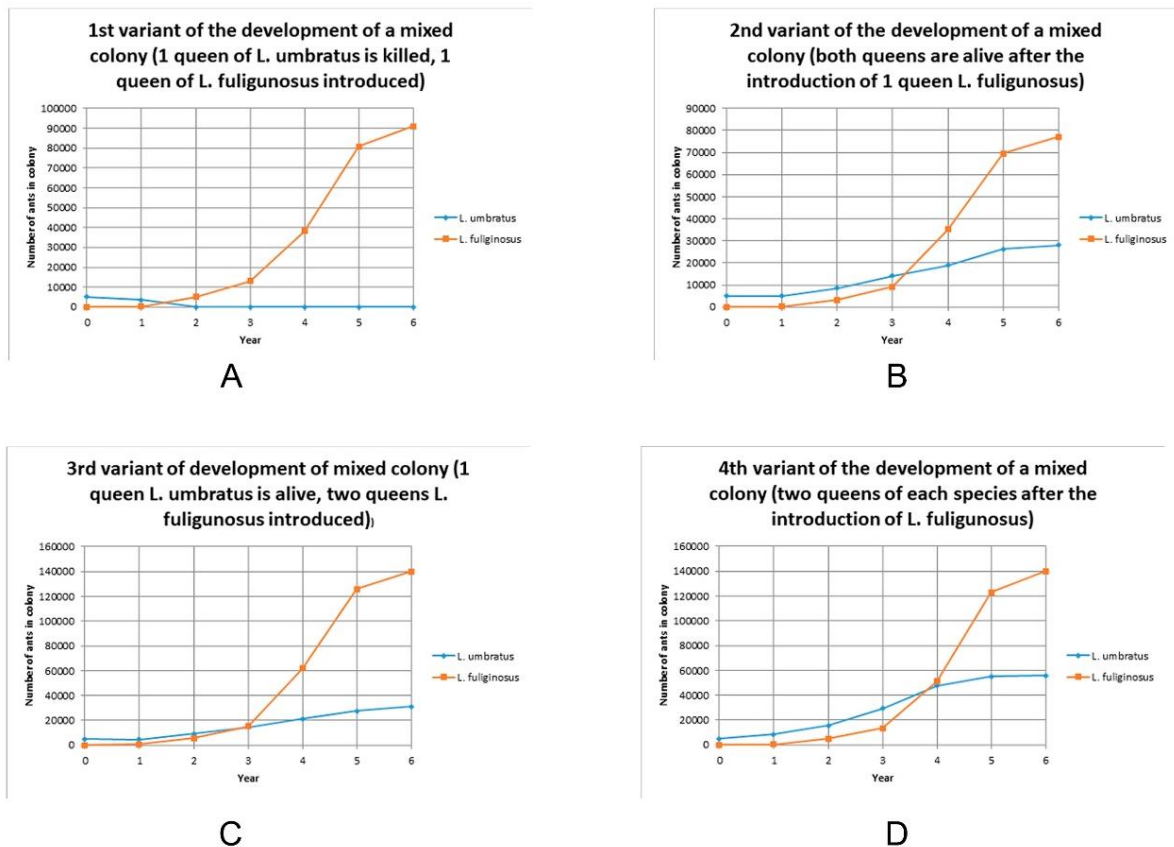


Figure 7. Results of mathematical modelling of the growth of the number of *L. umbratus* and *L. fuliginosus* workers in a mixed colony in four variants over a period of 6 years.

Table 1. Foraging activity in mixed colony 1, Rivne (Ukraine), results of Mann-Whitney pairwise comparison (with Bonferroni corrected p values)

Time of counting and ant species	10_ful	10_umb	20_ful	20_umb	30_ful	30_umb	40_ful	40_umb	50_ful	50_umb	60_ful	60_umb
10_ful		5.63E-05	0.0186	0.01429	0.000174	0.3461	1.64E-07	1	2.73E-06	1	3.54E-07	1
10_umb	5.63E-05		3.42E-09	1	3.44E-10	0.18	3.26E-10	5.55E-06	3.30E-10	3.78E-06	3.33E-10	2.69E-06
20_ful	0.0186	3.42E-09		5.23E-08	1	5.03E-07	0.000699	2.37E-05	0.1158	5.41E-06	0.005579	1.99E-05
20_umb	0.01429	1	5.23E-08		1.24E-09	1	1.02E-09	0.002598	1.02E-09	0.005271	1.03E-09	0.002284
30_ful	0.000174	3.44E-10	1	1.24E-09		3.19E-09	0.115	1.72E-08	1	4.78E-09	0.1386	1.62E-08
30_umb	0.3461	0.18	5.03E-07	1	3.19E-09		1.36E-09	0.6441	1.45E-09	1	1.32E-09	0.7194
40_ful	1.64E-07	3.26E-10	0.000699	1.02E-09	0.115	1.36E-09		2.28E-09	1	1.56E-09	1	2.08E-09
40_umb	1	5.55E-06	2.37E-05	0.002598	1.72E-08	0.6441	2.28E-09		3.74E-09	1	1.90E-09	1
50_ful	2.73E-06	3.30E-10	0.1158	1.02E-09	1	1.45E-09	1	3.74E-09		1.66E-09	1	3.29E-09
50_umb	1	3.78E-06	5.41E-06	0.005271	4.78E-09	1	1.56E-09	1	1.66E-09		1.51E-09	1
60_ful	3.54E-07	3.33E-10	0.005579	1.03E-09	0.1386	1.32E-09	1	1.90E-09	1	1.51E-09		2.01E-09
60_umb	1	2.69E-06	1.99E-05	0.002284	1.62E-08	0.7194	2.08E-09	1	3.29E-09	1	2.01E-09	

Note. The designations in the columns are as in Fig. 4C (counting time, minutes and species of ants), insignificant differences are highlighted in bold.

Table 2. Foraging activity in mixed colony 2, Rivne (Ukraine), results of Mann-Whitney pairwise comparison (with Bonferroni corrected p values)

Time of counting and ant species	10_ful	10_umb	20_ful	20_umb	30_ful	30_umb
10_ful		1,71E-08	0,4945	1,99E-08	0,1013	3,03E-07
10_umb	1,71E-08		7,36E-10	1	6,35E-10	0,005434
20_ful	0,4945	7,36E-10		5,96E-10	1	2,19E-09
20_umb	1,99E-08	1	5,96E-10		3,62E-10	0,1676
30_ful	0,1013	6,35E-10	1	3,62E-10		1,21E-09
30_umb	3,03E-07	0,005434	2,19E-09	0,1676	1,21E-09	

Note. The designations in the columns are as in Fig. 4D (counting time, minutes and species of ants), insignificant differences are highlighted in bold

It was found that ants live together in one nest, and there were 3 times less workers *L. umbratus* than workers *L. fuliginosus* (we counted 322 workers of *L. fuliginosus* and 89 workers of *L. umbratus*). Closer to the surface, under the stone, the number of workers of *L. umbratus* was somewhat higher (43 versus 19 *L. fuliginosus* workers). It is possible that the same ratio persist in the first colony, but the majority of *L. umbratus* workers do not come to the surface, but continue to forage underground.

Colony growth rate

Among the model variants of the development of a mixed colony of two species considered by us, the first variant seems unlikely (Fig. 7A). Already in the second year, most of the *L. umbratus* workers (about 90%) will die out due to natural causes, whereas in our case, a significant number of them (27 thousand in the Kyiv colony, 22-24 thousand in the two Rivne colonies) were observed. It is unlikely that a year ago the colony of *L. umbratus* was much more numerous, in the literature there is no data on colonies of this species with the number of workers more than 100 thousand. Therefore, it can be assumed that their population is replenished with new workers of *L. umbratus* due to the fact that the queen of this species remained alive. The second option-the preservation of both queens is most likely, since we have obtained a ratio of the number of workers of both species corresponding to real measurements (Fig. 7B). Most likely, both queens can remain alive in the conditions of spatial separation in the nest. In general, this is typical for species of ants with oligogyny, when the queens are in different chambers of the nest. Variants with several queens of each species are unlikely, but we hypothetically allowed them. Thus, in the third variant, one of the two queens of *L. umbratus* remained, but the colony also included two queens of *L. fuliginosus* (Fig. 7C). If the number of *L. umbratus* remained at the level corresponding to our observations, the number of *L. fuliginosus* was 2 times larger than the real one. Therefore, we consider this option as extremely unlikely. The same applies to the last, 4th variant (Fig. 7D), when there are two queens on each side. In this case, the population of *L. umbratus* will also double, which also does not correspond to our observations and makes this option even less

likely than the third one. From the simulation, therefore, the second option is most likely, with the preservation of one queen of each species as part of a mixed colony.

It is interesting to note that in each of the three colonies surveyed, the population ratio is approximately the same - the population of *L. fuliginosus* is 2-3 times larger than the population of *L. umbratus*, which also confirms the correctness of our mathematical model. In addition, this may be a confirmation that the population of *L. fuliginosus* is growing faster than the population of *L. umbratus*, and in all cases in approximately the same proportion. The accuracy of the model is also confirmed by the results of excavation of the nests.

According to the literature, the rate of colony growth can vary greatly, depending on the ant species, its level of social organization, as well as the stage of the colony's life cycle (its age). So, according to the theoretical calculations of Tschinkel (1988), for the fire ant (*Solenopsis invicta* Buren, 1972), for 7 years of its existence, its colonies are able to reach the level of 230 thousand workers. Although the theoretical calculations were generally consistent with the results of natural population counts, it was found that there are seasonal variations in the fire ant population. The maximum number in the colony was recorded in December, but by the period from March to August, it had fallen by half. This was due to the production of sexual individuals, which coincided with the natural decline of old workers. By midsummer, the production of new fire ant workers exceeds the mortality rate of old workers (Tschinkel, 1998). On the other hand, colonies older than 5.5 years may also experience a decrease in size. The 14-year-old fire ant colonies were significantly smaller than the 5.5-year-old colonies (Tschinkel, 1988). Such fluctuations are primarily due to the lack of food resources when they are monopolized by a single (invasive in the USA) ant species. Our calculations of colony growth were carried out for the period in the first 6 years of its existence, and we took into account that *L. fuliginosus* usually has monogyny, while the fire ant most often has polygyny. Therefore, and taking into account the temperate climate of Kyiv and Rivne, we calculated the growth of the colony at a slower pace. The relationship between the temperature regime

and the colony growth rate is also interesting. In the fire ant, in the first two months of its existence, small colonies grew at a faster rate than large colonies. The optimal temperature regime was 24-32°C, with worker mortality rates of 16% in small colonies and 10% in large ones. At 35°C, mortality increased to 18% in small colonies and 50% in large colonies (Porter, 1988). In our model, constant optimal growth conditions were taken into account. To a large extent, this is facilitated by the fact that the nests of *L. umbratus*, *L. fuliginosus* are cardboard structures located either underground in the wood remains, or in cavities inside withering or dry trees, i.e. their microclimatic conditions are more stable compared to nests dug in the ground thickness.

The leaf-cutting ant (*Atta bisphaerica* Forel, 1908) demonstrates high colony growth rates similar to the fire ant. The accounting was carried out from 2 months to 32 months after the mating summer. There was a growth from 121 worker ants to 65.000 individuals (Farias *et al.*, 2020). Such high colony growth rates are probably more characteristic of tropical ant species, which have a longer optimum period when the colony is actively growing. As for the early growth period of the colony, for another species of leafcutter, *A. texana* (Buckley, 1860), the number of workers on the 90th day of the experiment varied widely – from 10 to 110 workers (Marti *et al.*, 2015).

The dynamics of colony growth in harvester ants (*Pogonomyrmex barbatus* (Smith, F.)) is somewhat lower than in fire ants and leaf-cutting ants (Gordon, 1992). Direct excavations of their nests allowed us to establish that in colonies with an age of 1 year there were no more than 100 workers, 2 years – from 1.6 to 2.1 thousand, 3 years - from 2.0 to 6.6 thousand, 4 years - from 7.2 to 10.7 thousand. Another ant species, with a more primitive social organization (*Leptothorax curvispinosus* (Mayr)) shows a lower growth rate. Thus, in 4 months, the colonies grew from 57 to 70-123 workers (Evans and Pierce, 1995).

The effect of the number of queens in a colony on the rate of its growth is interesting. In the invasive species *Paratrechina fulva* (Mayr), the following results were obtained in the laboratory: 1 queen (monogyny) – 62 larvae in 53 days, 3 queens - 92 larvae in 46 days (oligogyny), 6 queens – 156 in 49 days

(polygyny) (Arcila *et al.*, 2002). In our four variants of mixed colony growth models, the variants of monogyny and oligogyny are considered, but the variant of monogyny seems more likely, since it explains the obtained real data on the abundance of *L. fuliginosus* and *L. umbratus*. The classical work of Sommer and Hölldobler (1995) provides data on the growth of *L. niger* colonies with different numbers of queens. A feature of *L. niger* is the establishment of colonies by pleometrosis, but in the end only one queen remains (soon after the first generation of workers). For 60 days of development of colonies with one queen there were 4 workers, with 2-8, 6-19 workers. It is worth noting that the effect of pleometrosis on colony growth is important for ant species with an independent colony founding. For *L. fuliginosus* and *L. umbratus*, which are a superparasite and parasite species, respectively, size of the host colony that their queens fall into may be important. If the colony of the host species is large enough, theoretically, the growth of the parasite population will also be accelerated in the presence of its two queens. We have shown this effect in our model, in variants 3 and 4.

As for foragers on trails, their number depends on the size of the colony. Studies conducted on laboratory colonies of *L. niger* of different sizes showed that colonies of up to 25 workers at the peak of foraging relied on a maximum of 5 foragers. Colonies of 50-75 workers relied on 13-14 foragers, and large colonies of 75-200 workers on 20 foragers (Mailleux *et al.*, 2003). This means that the larger the colony, the more foragers there will be on its trail. According to Zakharov (2015), foragers make up 13% of the total population of the entire colony. In our case, with the size of a mixed colony of one hundred thousand individuals (27 thousand and 72 thousand), about 13 thousand foragers of both species should be present on the trail.

Known cases of mixed colonies of L. fuliginosus and L. umbratus

The one of the first mention of the observation of mixed colonies of *L. umbratus* and *L. fuliginosus* is recorded by Lodeizen (1946). On September 30, 1939, at the base of the oak tree, he observed a mixed nest of *L. umbratus-fuliginosus* with a strongly dominant *L. umbratus* population. In 1940, the

population of *L. umbratus* declined, but they helped in all tasks, including visiting colonies of aphids. A year later, on March 13, 1941, the first entrance to the nest was opened, and the ants that died during hibernation were placed outside the nest by *L. fuliginosus* workers. The colony of *L. fuliginosus* became very numerous and spread in all directions from the oak. Very few *L. umbratus* workers were seen at the entrance to the nest that did not leave the nest. On April 22, 1941, the last *L. umbratus* worker was seen running through the underground passages. The interpretation of this study is as follows. The putative queen of *L. fuliginosus* enters the colony of *L. umbratus* ± June 5, 1939 (after the first period of the mating summer), kills the queen of *L. umbratus* and begins laying eggs ± June 15, 1939. *L. fuliginosus* occurred on August 8, 1939 and quite a few *L. fuliginosus* were already outside the nest on September 30, 1939. The last worker of *L. umbratus* is discovered 1 year and 11 months after the alleged murder of the queen of *L. umbratus*. Two years is the normal life expectancy for a small proportion of workers (5%). Interpretation: the queen of *L. umbratus* was killed in June 1939, but the workers of *L. fuliginosus* were in no hurry to kill the workers of *L. umbratus* ahead of time. They more or less allowed the *L. umbratus* population to become extinct according to their natural lifespan.

Thus, the total coexistence of the two species, according to Lodeizen (1946), lasted almost three years, two of which occurred in a comparable number of workers of *L. umbratus*. According to our calculations, it takes at least 5 years for both species to reach the population level of 27,000 *L. umbratus* workers and 72,000 *L. fuliginosus* workers. Even if we observed the same colonies in Kyiv and Rivne as described by Lodeizen (1946), in Moldova a mixed population was observed by one of the co-authors of this study (VS) for 5 years, i.e. during this time the population of *L. umbratus* must have been completely extinct, since at least 4 generations of workers were replaced. The possibility of preserving the queen of *L. umbratus* seems to us to be her spatial isolation from the queen of *L. fuliginosus* within the nest. Another option is if there were several queens of *L. umbratus* and one of them remained. This is possible if the colony of *L. umbratus*, during the penetration

of the queen of *L. fuliginosus*, was large enough and the second queen (or even the only one) of *L. umbratus* was left undetected by the queen of *L. fuliginosus*. We do not claim that such mixed colonies can co-exist for a long time, but we do admit that their existence is possible. Perhaps at some point the workers of *L. fuliginosus* will kill the queen of *L. umbratus*. The question of the existence of mixed colonies will help clarify future studies of these colonies in nature or their creation in the laboratory.

Our observations differed from those made by Lodeizen (1946). Unlike his data, no aphid visits were recorded – all *L. umbratus* workers went down the oak trunk "empty", while 90% of *L. fuliginosus* workers had their bellies swollen from the fall. On the other hand, we did not observe trophallaxis between the workers of both species, but they made antenna contact equally often. The very fact of the presence of *L. umbratus* workers on the surface already indicates a complete change in their lifestyle – from underground to active visits to trees.

The colony we observed was not a collection of two contiguous nests. This is evidenced by the presence of workers of both species, coming out of the same passages to the surface in the Kyiv colony. In addition, in the colony observed in Moldova, two species of workers moved through the underground passage. Another feature of the colony from Moldova was that only *L. fuliginosus* workers came to the forage tree. Therefore, it can be assumed that the workers of *L. umbratus* do not always go outside. We assume that while there are more *L. umbratus* workers in the mixed colony, *L. umbratus* workers are more likely to be underground. *L. fuliginosus* workers also can spend most of their time in underground passages, but when the ratio changes in favor of *L. fuliginosus*; *L. umbratus* workers can massively come to the surface. We observed hundreds of colonies of *L. fuliginosus*, of different sizes. However, no *L. umbratus* workers were ever seen on their trails. This indicates the uniqueness of the possibility of the existence of mixed colonies of two species.

In the usual situation, after the first appearance of *L. fuliginosus* workers, the workers of *L. umbratus* are killed, and this is the end of the coexistence of the two species. But it cannot be excluded that in some

situations, the killing of the *L. umbratus* worker population does not occur, and they can coexist side by side for several years. It is also impossible to exclude the presence of real mixed colonies with living queens of both species, but such cases are rare, only if the queen of *L. umbratus* remains alive for some reason.

The possibility of ants of different species visiting the same trail: Parabiosis

Parabiosis is the coexistence of two species of ants within the same nest. At the same time, both species use the same trails. Parabiosis was studied in detail in the works of Menzel *et al.* (2008), between the species *Crematogaster modiglianii* Emery, 1900 and *Camponotus rufifemur* Emery, 1900. It was found that the cuticle of ants of both species was covered with steroids, which were similar in structure to each other in ants of the same nest, but different between their different colonies. The hydrocarbon profile of both species was very different, with *Cr. modiglianii* workers tolerating *Ca. rufifemur* workers from their own colony, but attacking workers from other colonies. The authors suggest that there is a mutual transfer of the substance between *Cr. modiglianii* and *Ca. rufifemur*. In this case, *Ca. rufifemur* actively or passively acquires cuticular steroids from *Cr. modiglianii*, and the latter receives at least two cuticular hydrocarbons from *Ca. rufifemur*. Thus, the transfer of cuticular steroids is possible between pairs of parabiotic species. Interestingly, both species benefited from cohabitation - *Cr. modiglianii* is more efficient in finding trophic resources, whereas *Ca. rufifemur* is more efficient in nest protection (Menzel and Bluthgen, 2010). Slightly different results were obtained for another pair of species – *Cr. schmidtii*, *Ca. lateralis* (Stukalyuk and Radchenko, 2011). *Cr. schmidtii* workers invariably attacked even *Ca. lateralis* workers from "their" nest. The fundamental difference here is that both species did not live in the same nest, but in two independent colonies located not far from each other. Based on this, it can be assumed that the exchange of cuticular compounds is possible in the case when ant species live in mixed colonies. This case also includes the four colonies of *L. fuliginosus* and *L. umbratus* that we examined. Their co-existence is possible for a few years at least. In addition, there is no mutual benefit here – one of the

species parasitizes the other, and the preservation of the queen of *L. umbratus* is a hypothetical accident. Perhaps the long-term exchange of odors between these species contributed to a decrease in aggressiveness, up to the appearance of tolerance between them and the possibility of switching to a full-fledged parabiosis instead of the classic case of superparasitism. But this statement needs to be verified. It is worth considering that the profiles of cuticular hydrocarbons in *L. fuliginosus* and *L. umbratus* differ greatly, according to Holman *et al.* (2013). On the other hand, in *L. fuliginosus*, the profiles of cuticular carbohydrate channels are similar in workers from the same colony and differ between different colonies. The profiles of all body parts were similar, except for the chest-abdomen, head, and antennae (Toshiharu and Ryohei, 2002). Therefore, the possible mixing of hydrocarbon profiles between the two species can take place exclusively in the conditions of residence of workers of the two species for at least several years.

Pheromones of the genus Lasius

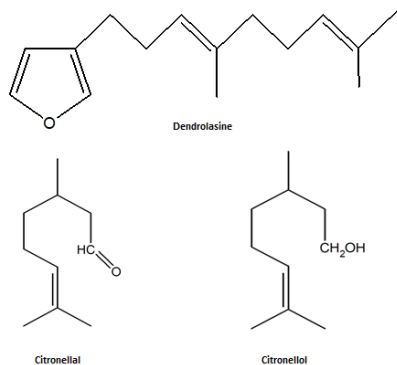
The main secretion of the mandibular glands of *L. fuliginosus* is dendrolasine (up to 86.4%, according to Bernardi *et al.* (1967), Tab. 3), in *L. umbratus* it is citronellol (85%, an alarm pheromone) and citronellal (3.4%, a protective agent, according to Blum *et al.*, 1968). These substances act as anxiety pheromones. For *L. fuliginosus*, the main abdominal extracts were: n-undecane (18.9%), n-tridecane (12.8%), dendrolasine (17%), pentadecane-2-one (35%), and heptadecane-2-one (12.4%).

Dendrolasine is a terpene with furan ring belongs to the sequiterpenes.

Citronellal (3,7-dimethyl-6-octenal) is an aldehyde, a derivative of terpenoids (with the smell of lemon).

Citronellol (3,7-dimethyl-6-octen-1-ol) is an alcohol derivative of terpenoids (with a rose smell).

Dendrolasine and citronellol belong to different terpenes, dendrolasine has a ketone active group, and citronellol has an alcohol group. i.e. both terpenes, but with different functional groups, in *L. fuliginosus* this is a terpene with ketone, and in *L. umbratus* terpene with an alcohol group. Citronellol and citronellal are two transitional forms of the same compound, only one is more stable and numerous.



Henecane (undecane) - a liquid hydrocarbon with the chemical formula $\text{CH}_3(\text{CH}_2)_{10}\text{CH}_3$, has 159 isomers is the main component in the composition of abdominal secretions in *L. alienus*, *L. carniolicus*, *L. flavus*, whereas *L. fuliginosus* has heptadecane-2-one as the main component. Heptadecane-2-one is a ketone derivative of alkane hydrocarbons of the same series as Henecane. The composition of abdominal secretions in *L. fuliginosus* is generally qualitatively different from the rest of the aforementioned *Lasius*. Only undecane and n-tridecane in the composition of abdominal secretions are related to *L. fuliginosus* with other *Lasius* species. Also in the composition of abdominal secretions in this species, ketone derivatives of hydrocarbons and cis-citral and trans-citral predominate.

For *L. niger*, acetates are widely represented in the composition of abdominal secretions, especially given that the main component is 1-dodecyl acetate (Tab. 3). In the composition with other acetate derivatives of hydrocarbons, 1-decyl acetate, 1-hendecyl acetate, 1-tetradecyl acetate, 1-hexadecyl acetate, 1-octadecyl acetate forms a specific composition that differs from other *Lasius* species, despite the presence of a very common Henecane. Also, the presence of a small amount of alcohol derivatives of decane series hydrocarbons makes the composition of abdominal secretions in *L. niger* very special.

For *L. alienus*, the composition of abdominal secretions is mainly represented by long-chain hydrocarbons in the presence of ketone derivatives of these hydrocarbons and a minimum content of 1-hexadecyl acetate (Tab. 3). There is also a specific component of methyl n-hexadecanoate-the methyl ester of hexadecanoic acid, which is not found in other *Lasius* species.

For *L. carniolicus*, the composition of abdominal secretions is represented by both

the hydrocarbons hendecane, 3-methylhendecane, tridecane, and a wider content of ketone derivatives of hydrocarbons 2-tridecanone, 2-pentadecanone, 3-tetradecanone, and 3-hexadecanone of similar composition than that of *L. alienus* (Tab. 3). In addition, the composition contains Citronellal, geranyl citronellal, which makes the composition of abdominal secretions different from other *Lasius* species, but similar to the composition of the mandibular gland in *L. fuliginosus*, and the presence of a ketone and an aldehyde derivative of low - mass hydrocarbons-6-methyl-5-hepten-2-one, 2,6-dimethyl-5-hepten-1-al is not characteristic of the composition of abdominal secretions other *Lasius* species, but again characteristic of the composition of the mandibular gland in *Lasius fuliginosus*. The minimum content of solid terpene alcohol-geranylgeranial is a feature of the composition of abdominal secretions in *L. carniolicus*.

The composition of abdominal secretions in *L. flavus*, although represented by the main component of hendecane, has a large number of aldehydes 4-hydroxyoctadec-9-enolide, 1-nonanal, w-aldehyde of 4-hydroxynonanolide, 4-hydroxyoctadecanolide, which makes this composition special (Tab. 3). It also contains a specific terpene-2,3-dihydrofamesal. A common component of the composition of abdominal secretions in *L. flavus* and *L. carniolicus* is citronellal.

L. umbratus has one component in common with the composition of abdominal secretions in *L. fuliginosus*, it is tridecan-2-one, as well as the composition of mandibular gland, abdominal secretions, *L. fuliginosus* hind gut extract contains many ketone derivatives of hydrocarbons (Tab. 3). The chemical composition relationship is known to be traced between *L. fuliginosus* and *L. umbratus* only by these compounds. At the same time, dendrolasine and citronellol, citronellal is quite close (terpenes), which can cause similar behavioral reactions of both species in a mixed colony in case of danger.

An interesting study was conducted in 1967 by Hangartner. Traces of *L. fuliginosus*, as well as three other species of the genus *Lasius*: *L. niger*, *L. flavus*, and *L. emarginatus* were studied. One of the conclusions of the work was that the trace substances of *L. fuliginosus* have no dependence on either the colony or the caste.

Table 3. Trace pheromones and alarm pheromones used by ants of different species of the genus *Lasius* (literature data)

Ant species and gland	Chemical name	Reference
<i>Lasius fuliginosus</i> , trail pheromones	Hexanoic acid Heptanoic acid Octanoic acid Nonanoic acid Decanoic acid	Huwyler <i>et al.</i> (1973,1975)
Mandibular gland	Dodecanoic acid Dendrolasine 6-methyl-hept-s-en-2-one, perillen, cis-citral, trans-citral, dendrolasin, farnesal	Quilico <i>et al.</i> 1956, 1957a
Abdominal secretions (possible from Dufour's gland)	Pentadecane-2-one, dendrolasin, n-undecane, n-tridecane, heptadecane-2-one (main components), cis-citral, trans-citral, tridecane-2-one (minor components).	Bernardi <i>et al.</i> , 1967
Hindgut extract	3,4-Dihydro-8-hydroxy-3-methylisocoumarin (mellein), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Kern <i>et al.</i> , 1997
Alarm pheromones	Decane, n-Undecane	Dumpert, 1972
<i>Lasius umbratus</i> , mandibular gland	Citronellal - releaser of alarm Citronellol - defensive secretion n-hendecane, n-tridecan-2-one	Blum <i>et al.</i> , 1968; Quilico <i>et al.</i> , 1957b
<i>Lasius niger</i> Abdominal secretions (possible from Dufour's gland)	Hendecane, 1-dodecyl acetate (main components), tridecane, heptadecene, heptadecane, nonadecane, 1-decyl acetate, 1-hendecyl acetate, 1-tetradecyl acetate, 1-hexadecyl acetate, 1-octadecyl acetate, 1-hendecanol, 1-dodecanol, 1-hexadecanol (minor components).	Bergstrom and Lofqvist, 1970
<i>Lasius alienus</i> Abdominal secretions (possible from Dufour's gland)	Hendecane (main component), tridecane, nonadecene, heneicosane, 2-tridecanone, 2-pentadecanone, 2-nonadecanone (considerable amounts), decane, dodecane, pentadecane, heptadecene, heptadecane, 2-heptadecanone, methyl n-hexadecanoate, 1-hexadecyl acetate (minor components).	Bergstrom and Lofqvist, 1970
<i>Lasius carnolicus</i> Abdominal secretions (possible from Dufour's gland)	Hendecane (main component), 2-tridecanone, 2-pentadecanone (considerable amounts), 3-methylhendecane, tridecane, 3-tetradecanone, 3-hexadecanone (minor components)	Bergstrom and Lofqvist, 1970
Head	Citronellal, geranylcitronellal (main components), 6-methyl-5-hepten-2-one, 2,6-dimethyl-5-hepten-1-al,	

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	geranylgeranial (minor components).	
<i>Lasius flavus</i> Abdominal secretions (possible from Dufour's gland)	Henecane (main component), 4-hydroxyoctadec-9-enolide, 1-nonanal, w-aldehyde of 4-hydroxynonanolide, 4-hydroxyoctadecanolide (considerable amounts), decane, tridecene, tridecane (minor components).	Bergstrom and Lofqvist, 1970
Head	Citronellal, 2,3-dihydrofamesal.	

All the studied species accumulate their trace substance in the ampoule of the rectum. *L. fuliginosus* workers can interpret all traces of *L. emarginatus*, *L. niger*, with the exception of the trace substance of *L. flavus*, but the secrets in their own rectal ampoules cannot be understood by any of the other species studied. Thus, the trace substance of *L. fuliginosus* is very species-specific. This specificity does not depend on the concentration of the test solution.

The features of the interaction of *L. fuliginosus* and *L. umbratus* within a mixed colony should not necessarily be determined by the similarity of alarm pheromones and trace substances. Włodarczyk (2012) investigated the levels of aggressiveness of ants from a mixed colony of *Formica sanguinea* and *F. polyctena* to ants of the same species from single-species colonies. It was found that *F. polyctena* workers behaved more aggressively towards ants from a mixed colony, but no such aggression was detected in *F. sanguinea*. As a result, the ants of both species clearly distinguished between conspecific and allospecific ants from the mixed colony. The author concludes that as a result of social interactions, both species exchanged cuticular hydrocarbons, which led to some adjustment of their recognizable labels. Thus, the exchange of cuticular hydrocarbons can also be one of the predictors of the occurrence of mixed colonies. More recent work by Włodarczyk & Szczepaniak (2014) confirmed the hypothesis of cuticular hydrocarbon exchange. Chemical analysis of hexane extracts from the surface of ants' bodies confirmed the interspecific exchange of alkenes and alkanes with a methyl-branched chain. It is noteworthy that *F. sanguinea*, like the two species we studied, is also a parasite ("slave owner"). It is possible that parasitic and slave-owning species have adaptations that allow them to easily interact with the workers of the host species by exchanging cuticular hydrocarbons. It is also known that in

the hierarchical structure of ant associations, some subordinate species can recognize cuticular hydrocarbons of the dominant species: avoid the area with the smell of cuticular hydrocarbons of the dominant species, while the dominant species either ignore or are attracted by the smell of cuticular hydrocarbons of the subordinate species (Binz *et al.*, 2014). The authors conclude that ants of different species use heterospecific cues to avoid or seek out competitors, and that their response depends on their rank of dominance in the local community.

Conclusion

We found 4 mixed colonies that contained almost the same ratio of workers of *L. umbratus* and *L. fuliginosus* (1: 2 or 1: 3). The results obtained corresponded to the constructed model of colony growth. Presumably, such mixed colonies can exist, provided that the queen *L. umbratus* is preserved (or isolated) after the queen *L. fuliginosus* has entered the colony. Apparently, such mixed colonies are short-lived, at least in half of the cases (colonies from Kyiv and Transnistria) their life cycle ended with the death of the entire population of both species of ants. From this we can conclude that the coexistence of such mixed colonies does not exceed a period of 5 years. The first 3-4 years, such colonies grow, then the queens of both ant species are most likely to die, and the mixed colony dies out. Workers of *L. umbratus* do not take part in transporting and hunting prey, outside of the feeding area, but simply move along trails between the central and auxiliary nests. We established the presence of *L. umbratus* workers in colony 2 from the city of Rivne (Ukraine) in three distant nests, one central and two auxiliary nests. At the same time, workers of *L. umbratus* can feed on prey. It has been established that workers of *L. umbratus* can participate in the defense of the nest, leaving the passages when the enemy approaches the nest itself, and moving along the paths when

mobilizing against the enemy. It is possible that the foraging area of a mixed colony in the first years of its existence (while the colony is growing) includes 1. aboveground part, where *L. fuliginosus* mainly forages, and 2. underground part, where *L. umbratus* forages. In the future, it is necessary to conduct an experiment with the creation of a mixed colony in laboratory conditions, with the possibility of isolating the queen *L. umbratus*. It is also necessary to test the hypothesis of possible mixing of cuticular hydrocarbons between the population of the mixed colony in order to identify the reason for the tolerance of workers *L. umbratus* and *L. fuliginosus* to each other.

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