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Original Article

Nest modifications by the acorn ant *Temnothorax crassispinus* (Hymenoptera: Formicidae)

Sławomir Mitrus

Abstract

Many ant species construct nests and during the process considerably influence the environment such as by changing soil structure and creating new habitat for other species. However, other ant species dwell in ready-for-use cavities. Ants of the genus *Temnothorax* inhabit small cavities such as acorns and under rocks, but under natural conditions, good nest sites are limited resources. During field and laboratory experiments, I studied how the acorn ant *Temnothorax crassispinus* (Karawajew, 1926) modifies nesting sites. *Temnothorax crassispinus* is a forest species, which typically lives in cavities in fallen twigs and acorns; colonies usually number from a few dozen to about 200 workers. Although it is known that they prefer narrow entrances, in a field experiment, a similar proportion of artificial nest sites with narrower and wider entrances were inhabited, and most colonies decreased entrance sizes. Similarly, in laboratory experiments, colonies decreased entrance sizes. Colonies used more sand grains when sand was placed closer to the nest entrance than when it was farther; however, I found no relationship between the number of grains of sand used for such modifications and colony size, and the presence of other colonies in the same Petri dishes did not affect entrance-size reduction. Other experiments showed that the ants can enlarge the nest cavity, but this depends on the material filling the cavity, and that the ants are able to dig nest chambers just in soil. Thus, acorn ants can modify and create nest sites, and may thus also modify the environment.

Key words: *Temnothorax crassispinus*, nest site, entrance modification, nest cavity, cavity-nesting ant.

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Sławomir Mitrus, Institute of Biology, University of Opole, Oleska 22, 45-052 Opole, Poland.
E-mail: smitrus@uni.opole.pl

Introduction

Animals may modify their environment adaptively. Typical examples of species considerably changing the environment include beavers (*Castor* spp.), which construct dams and thus alter the distribution and the abundance of many organisms in the larger area (Jones & al. 1994), but also social insects, e.g., ants and termites, due to the impact of the processes of soil formation when creating nests (Jones & al. 1994, Frouz & al. 2003, Frouz & Jilková 2008, Whitford & Eldridge 2013, Farji-Brener & Werenkraut 2017). During nest construction, ants bring sediment from lower soil layers to the surface, dig tunnels, and mix the soil, thus increasing aeration and changing nutrient concentration (Whitford & Eldridge 2013). For ants, nests provide shelter and protection from extreme temperatures, provide a refuge against desiccation and predators, and ensure optimal conditions for the brood (Blüthgen & Feldhaar 2009).

Many ant species do not construct permanent nests, but utilize available cavities, as nest construction is costly in terms of work time and energy (Mikheyev & Tschinkel 2004, McGlynn 2012). Some ant species more frequently inhabit nest cavities with certain volume (e.g., Herbers & Banschbach 1995, Novais & al. 2017), but ants can also modify available cavities. For example, *Leptothorax acervorum* (Fabricius, 1793) – an ant typically nesting in decaying logs, detached branches and under barks (Czechowski & al. 2012) – can build loose partitions from wood fragments inside the cavity (Sudd & Franks 1987), *Temnothorax nylanderi* (Förster, 1850), can enlarge the nest cavity (Foitzik & Heinze 1998), and *T. rugatulus* (Emery, 1895) and *T. albipennis* (Curtis, 1854) – which nest in rock crevices or under stones – build walls to create nests or improve nest quality (Aleksiev & al. 2007a, b, c, DiRienzo & Dornhaus 2017). The nest entrance is very important, as it can facilitate access by predators or pathogens, influence the social interface, and serve ventilation purposes (Halboth & Roces 2017, Lehue & Detrain 2019). The diversity of nest entrance sizes could affect the structure of ant communities (e.g., Powell & al. 2011, Jiménez-Soto & Philpott 2015). For example, Novais & al. (2017) found that body size of ants dwelling in twig cavities was positively correlated with the diameter of the nest entrance; however, ants are also able to decrease entrance size (Pratt & Pierce 2001).

The main objective of this study was to investigate nest site modifications by the acorn ant *Temnothorax crassispinus* (Karawajew, 1926) – widely distributed in forests in Central Europe. *Temnothorax* is one of the most species-rich ant genera distributed in the Holarctic region, Sub-Saharan Africa, and in Mesoamerica (Czechowski & al. 2012, Prebus 2017). Colonies of the ants are small, typically containing several dozen workers. Some species inhabit cavities in dry branches of living trees; others inhabit cavities under stones, in rocks, in fallen twigs, under bark, in acorns, but also among leaf litter, and in the ground (Czechowski & al. 2002, 2012, Prebus 2017). The number of good quality nest sites for *Temnothorax* ant colonies are usually limited (Herbers 1989, Herbers & Johnson 2007), but the ants can select a nest from among various sites if available. For example, ants prefer nest cavities with narrow entrances, which are easier to defend (Pratt & Pierce 2001, Franks & al. 2003, Pratt 2010). *Temnothorax* ants are able also to choose a superior site even if it is located at a greater distance than a nearby nest site of lower quality (Franks & al. 2008). They are also able to decrease entrance size by adding rims of soil and leaf litter (Pratt & Pierce 2001), build walls in preformed cavities to improve nest sites (Aleksiev & al. 2007a, b, c), and in the laboratory they often decrease entrances to an artificial nest cavity by using, e.g., pieces of wood from natural nest site, if available (S. Mitrus, unpubl.). This study posed the following questions: (1) Are acorn ants inhabiting and modifying worse nest sites under high nest site competition? (2) Do modifications of entrances and cavities depend on colony size, and other factors, such as the presence of other colonies? (3) Are acorn ants able to dig a nest cavity directly in soil? For this study, I performed field and laboratory experiments.

Materials and methods

Five experiments were performed, one field and four laboratory ones, using colonies of the acorn ant *Temnothorax crassispinus*. The ant is present throughout Central and Eastern Europe and is widely distributed in Poland. *Temnothorax crassispinus* lives in light coniferous and mixed forests (Seifert 2007, Czechowski & al. 2012). Workers of the ant are small, only 2 - 4 mm in length (Seifert 2007, Czechowski & al. 2012). Colonies typically number from a few dozen to about 200 workers. Their nests are located in the litter layer, mostly in acorns and small sticks (Seifert 2007, Białas & al. 2011, Czechowski & al. 2012, Myczko & al. 2018), where larvae of other insects have bored cavities (Foitzik & al. 2004, Myczko & al. 2017). To determine the ant species, I used the key in Radchenko & al. (2004).

Experiment 1 (a field experiment): Are acorn ants inhabiting and modifying worse nest sites under high nest site competition? Ninety-six artificial nests made of beech woodblocks measuring 7.5 cm × 2.0 cm × 2.0 cm were used. Each woodblock was drilled lengthwise to form a 4 mm hole, which was tightly closed on one side with a beech plug (Fig. 1A, see also Appendix, as digital supplementary material to this article, at the journal's web pages); after calculating the reduction in the length of the drilled cavity due to the beech plug, the final cavity volume was approximately 850 mm³. Because ants prefer cavities with narrower entrances (e.g., Pratt 2010), the hole on the other side typically is partially closed by a splinter during experiments in which such artificial nest sites are used; such nest cavities are willingly accepted by *Temnothorax* ants (e.g., Foitzik & al. 2003, Mitrus 2015). However, during the experiment, the hole was reduced (a wood splinter filled a half of the hole on the other side) for only half (i.e., 48 ones) of the artificial nests (= sites with "narrow" entrances) (Fig. 1B). The remaining nest sites (48) had no such splinter; thus, their entrances were not reduced (= sites with "wide" entrances) (Fig. 1A). It was predicted that cavities with "narrower" entrances will be preferred by ants (namely, more such nest sites will be inhabited), and "wide" entrances will be reduced by ants.

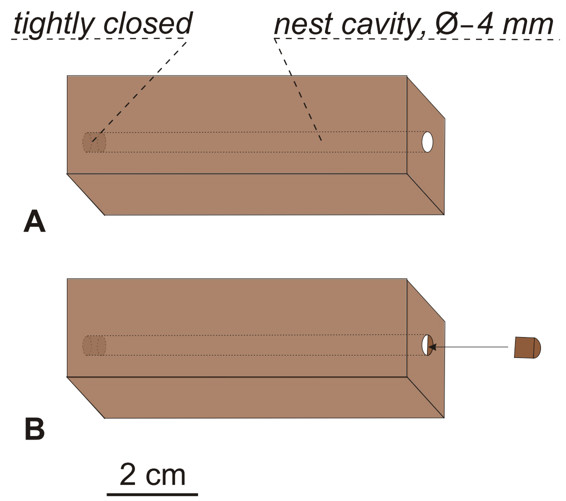

Fig. 1: Nest design in the field: woodblock drilled lengthwise to form a 4 mm hole, tightly closed on one side with a beech plug creates a nest site with "wide" entrance (A). For half of the study nest sites, the hole on the other side was additionally partially closed by a splinter to create a nest site with "narrow" entrance (B). The final cavity volume was approximately 850 mm³.

The experiment was conducted in mixed forest with a dominance of pine (Opole district, Poland, GPS: 50°32'46" N, 18°06'35" E). The presence of *Temnothorax crassispinus* colonies was confirmed in the area prior to the start of the experiment. On 4 April 2018, the artificial nests were placed in the field. The nests were attached to ca. 16 cm sticks poked into the ground; distance between nests was ca. 75 cm. About three and a half months later (17 July 2018), the artificial nests were collected and, in each case, it was noted if the entrance had been modified. However, one nest was pushed into the soil and its

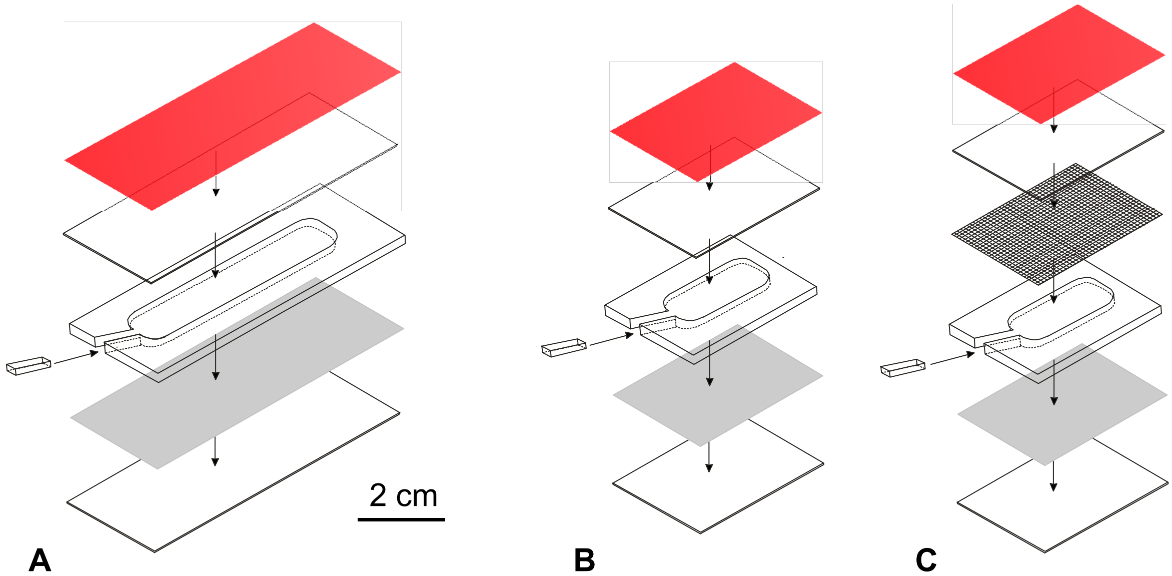

Fig. 2: Nest design in laboratory experiment: a cavity between a piece of cardboard and a microscope slide (76 mm × 26 mm), kept apart by a Plexiglas frame (3 mm thick), coated with a piece of a red translucent filter (A). A small piece of Plexiglas in the entrance reduced the entrance by 50%. The volume of the cavity was ca. 1760 mm³. For one experiment (see text for the details), a cavity was smaller: it was between a piece of cardboard and half of a microscope slide (38 mm × 26 mm) (B); the cavity volume was ca. 860 mm³. Additionally, in the experiment, half of nest sites had additionally a piece of metal mesh between the Plexiglas frame and the upper slide (C); thus, when the entrance to such nest was closed, and the upper slide removed, through the metal mesh, pheromones could spread, but ants were not able to leave the nest cavity.

entrance was blocked by soil; this nest site was thus not used in analyses. In the laboratory modified entrances were measured to the nearest 0.1 mm: The diameter of the hole was measured, unless the hole was elliptical, in which case – the longest diameter of the hole and the orthogonal diameter (cf. Pratt & Pierce 2001). The nest sites were then carefully opened, and the ants were captured with an aspirator and counted. In cases of cavities containing only workers (i.e., where no eggs or pupae were found), these colonies were classified as "nests containing only a few individuals".

Laboratory conditions during experiments: For laboratory experiments (Experiments 2 - 5, see below), ant colonies near Opole (Poland, GPS: 50°34'50" N, 18°11'00" E) were collected. Previous observations indicated that in a laboratory *Temnothorax* ants can use small pieces of wood from the nest to reduce entrances to nest sites (S. Mitrus, unpubl.). Thus, for the experiments, colonies in acorns only were collected: From such cavities it is easier to collect ants without any pieces of nest sites, which could be used later by workers for entrance reduction. In the laboratory, the acorns were carefully opened, then ants captured with an aspirator and counted. During the study, all used colonies contained brood. Each ant colony was placed in a separate square Petri dish (10.2 cm × 10.2 cm × 1.9 cm) having – with the exception of Experiment 5 (see below) – a thin plaster base and an artificial nest chamber placed on top. The Petri dishes with ant colonies were kept in a thermostatic cabinet. A daily cycle was maintained throughout the experiments: 10 h of dark ("night") in a temperature of 17 °C, and 14 h of light ("day") in 27 °C (the same as artificial summer conditions previously used in experiments on *T. nylanderi*, e.g., Foitzik & al. 2003). Ants were fed twice a week with frozen Dubia roach (*Blaptica dubia*) (length: ca. 10 mm), honey, and *ad libitum* water.

Experiment 2 (a laboratory experiment): Does distance between nest site and source of sand affect nest entrance modification? On 5 September 2018, 44 acorns containing ant colonies were collected (14 queenless, 28 containing one queen, and two with two queens each; workers: 12 - 282, median 88). For the experiment, 40 colonies without a queen or with one queen were chosen and transferred to Petri dishes. Each dish contained an artificial nest site: a cavity between a piece of cardboard and a microscope slide (i.e., 76 mm × 26 mm), kept apart by a Plexiglas frame (3 mm thick), coated with a piece of a red translucent filter from above; the volume of the cavity was ca. 1760 mm³ (cf. Mitrus 2015). As these ants prefer cavities with narrower entrances, and such cavities are inhabited faster (S. Mitrus, unpubl.), a small piece of Plexiglas was put in the entrance; the nest site entrance was then reduced by 50% (from 3 mm × 3 mm to 1.5 mm × 3 mm, cf. Fig. 2A).

After about 24 hours, the Petri dishes were checked. Two colonies had not inhabited the nest sites, so they were not considered in the experiment. Thus, during the experiment, 38 colonies were used (12 queenless, and 26 queenright; workers: 13 - 282, median 90). The pieces of Plexiglas used to reduce entrance size were removed. Next, colonies were randomly divided into two groups, and ca. 1.5 ml of dry sand added (size of grains: 0.4 - 0.8 mm, mean = 0.60, SD = 0.19, based on a random sample of 118 grains; grains that passed through an 0.8 mm sieve, but not through a 0.4 mm one): for one group, about 3 cm from the nest cavity entrance; for the second, about 10 cm from entrance. After five days, the grains of sand used by ants for entrance-size reduction were collected: grains just at the entrance, or directly near the entrance. As ant colonies always used a mix of differently sized grains, the number of grains of sand used for nest entrance modification was taken as the result. It was predicted that shorter distance would lead to a larger number of grains used.

Experiment 3 (a laboratory experiment): Does presence of other ant colonies affect nest entrance modification? Ant colonies avoid sites that are too close to competing colonies (Pratt 2010). Under natural conditions, however, because of high density (for *Temnothorax crassispinus* locally even several colonies per square meter; Strätz & Heinze 2004, Białas & al. 2011), cavities near other colonies are sometimes the only ones available. For the experiment, colonies from two locations were used (distance between the locations was more than 30 km; GPS: 50°51'54" N, 18°07'33" E, and 50°34'50" N, 18°11'00" E). *Temnothorax* ant colonies can present polydomy (during the active season one colony at the same time can use more than one nest; Strätz & Heinze 2004, Debout & al. 2007), so collecting colonies from two distant locations was necessary to ensure that colonies placed in one Petri dish (see below) were not closely related. First, on 10 September 2018, 43 colonies were collected (12 queenless, and 31 queenright; workers: 12 - 278, median 68), and transferred to Petri dishes with artificial nest cavities. For these colonies, an artificial nest site was a cavity between a piece of cardboard and a half of a microscope slide (i.e., 38 mm × 26 mm), kept apart by a 3 mm thick Plexiglas frame, and coated with a piece of a red translucent filter (Fig. 2). Additionally, a piece of metal mesh was put (woven stainless wire mesh: wire diameter 0.2 mm, hole size: 0.31 mm) between the Plexiglas frame and the upper slide (Fig. 2C). Next, on 12 September, ants at the second location were collected (37 colonies, 7 without queen and 30 with one queen; workers: 12 - 288, median 87). They were transferred to Petri dishes with artificial nest sites as in Experiment 2. The next day, it was checked if the colonies inhabited nest sites (five of them had not). Colonies (32) in nest sites and foraging workers were then carefully transferred to new Petri dishes, and randomly divided into two groups:

– treatment group: to each dish a nest site with the ant colony from the other location, collected on 10 September, was transferred (see above); however, previously, the entrance to the nest was fully closed, and the upper slide was removed (cf. Fig. 2C). Thus, through the metal mesh, pheromones could spread, but ants were not able to leave the nest cavity;

– "control group": to each dish an empty nest site with metal mesh and closed entrance was transferred.

The pieces of Plexiglas used to reduce entrance size of the focal colonies' nests were removed, and dry sand (ca. 1.5 ml, size of grains: 0.4 - 0.8 mm) was added near the centre of the Petri dish; the distance of the sand from the nest entrance was about 5 cm. After 24 hours, the grains of sand used by ants for entrance size reduction were collected (grains just in the entrance, or directly near the entrance). The sand was collected after 24 hours, as the majority of sand grains are transported during the first day after cavity dwelling, and when alerted, colonies should quickly reduce entrances to cavities. Thus, it was predicted that presence of other colonies would affect nest entrance modification, that is, colonies with dwelling in cavities near other ant colonies will use more grains of sand. In all cases, the two colonies placed in the same Petri dish were similar in size.

Experiment 4 (a laboratory experiment): Do larger colonies remove more material filling the nest cavity? For the experiment, 40 colonies collected on 17 September were used (10 queenless and 30 queenright; workers: 22 - 268, median: 114). They were randomly divided into two groups and transferred to Petri dishes with artificial nest cavities like in Experiment 2; however, the cavities were filled ca. 80% with:

– the first group: small pieces of fresh wood (made of twigs without ant colonies collected in the forest; the twigs were ground down by hand, length of the pieces: ca. 1 - 8 mm);

– the second group: dry sand (size of grains: 0.4 - 0.8 mm).

Thus, as the volume of the artificial nest cavity was ca. 1760 mm³, and ca. 80% of the volume was filled, the remaining volume of the cavity was ca. 0.35 ml. After four days, pieces of wood / grains of sand removed from the nest cavities by ants were collected. The volume of the wood pieces (up to about 0.25 ml) and the counted grains were evaluated. It was predicted that larger colonies would remove more pieces of wood / grains of sand.

Experiment 5 (a laboratory experiment): Are acorn ants able to dig a nest cavity directly in soil? Nineteen colonies from Experiment 2 were used (see above; 7 queenless and 12 queenright colonies, workers: 13 - 255, median 92). First, clean, dry sand and wet soil were added (from a gardening shop, soil used for plant cultivation) to empty Petri dishes: mix 1:1, light tamped down, thickness of the layer of sand and soil about 10 mm. Three small, flat stones (surface of each of these stones ca. 4.5 - 18 cm²) were placed on the surface of the layer in each dish. Next, the colonies were transferred without nest cavities to the Petri dishes with the layer of sand and soil. The experiment started on 11 September. After ten days, it was noted where the colonies were dwelling. It was predicted that if there is no good-quality cavity for a nest, the acorn ants will create a nest cavity in the soil, under the stones.

Data analysis: The chi-square test was used to test if under high nest-site competition acorn ants inhabited artificial nest sites with "narrow" vs. "wide" entrances in similar proportions (Experiment 1, the field experiment). For data obtained in the experiment, a two-way analysis of variance was also used: to check if number of workers was similar in colonies collected from nest sites with "narrow" vs. "wide" entrances and to compare sizes of queenright vs. queenless colonies; assumptions for the data distribution were checked before the analysis using statistical tests for normality and homogeneity of variance and using appropriate graphs. Sizes of modified entrances between nest sites with "narrow" vs. "wide" entrances were compared using a Mann-Whitney U test, as distributions of the data were skewed.

For the data obtained in the laboratory experiments, general linearized models (GLM) were used to compare the number of grains of sand used by ant colonies (groups for which sand was closer vs. farther from nest entrances; Experiment 2). The GLM included the number of workers in a colony as a continuous predictor. Similarly, data for treatment group (with presence of other colonies) vs. control group (Experiment 3) were analysed; however, as the distributions of numbers of workers were right skewed, the data were transformed before the analysis using square root transformation.

Spearman rank correlation was used to assess the relations between the number of workers in a colony and the volume of removed pieces of wood, and between the number of workers in a colony and the removed grains of sand (Experiment 4).

All above-mentioned statistical analyses were carried out using the software package Statistica, ver. 13 (Dell Inc. 2016). The threshold for significance was $p = 0.05$ throughout. All probability values shown are two-tailed.

Results

Experiment 1 (a field experiment): Are acorn ants inhabiting and modifying worse nest sites under high nest site competition? Colonies of the ant *Temnothorax crassispinus* (10 queenless, 25 queenright; 13 - 174 workers, median 52) were discovered in 34 of 47 nest sites with "narrow" entrances, and 35 of 48 sites with "wide" entrances (12 queenless, 22 queenright; 10 - 88 workers, median 38.5; Fig. 3); I found no other invertebrates in the sites. In July 2018, contrary to prediction, a similar proportion of sites with "narrow" and "wide" entrances were inhabited by the ant colonies ($\chi^2 = 0.00$, df = 1, $p = 0.95$; the analysis concerned the number of cavities inhabited by ant colonies – both queenright and queenless – in comparison with cavities where ant colonies were not found; namely: both empty cavities and those with only a few individuals, without brood).

There was no difference in proportion of queenright and queenless colonies in nest sites with "narrow" vs. with "wide" entrances ($\chi^2 = 0.36$, df = 1, $p = 0.55$). Number of workers in queenright colonies was higher than in queenless ones ($F_{1,65} = 21.43$, $p < 0.0001$), but there were no differences between colonies inhabiting sites with "wide" vs. "narrow" entrances ($F_{1,65} = 21.43$, $p = 0.10$).

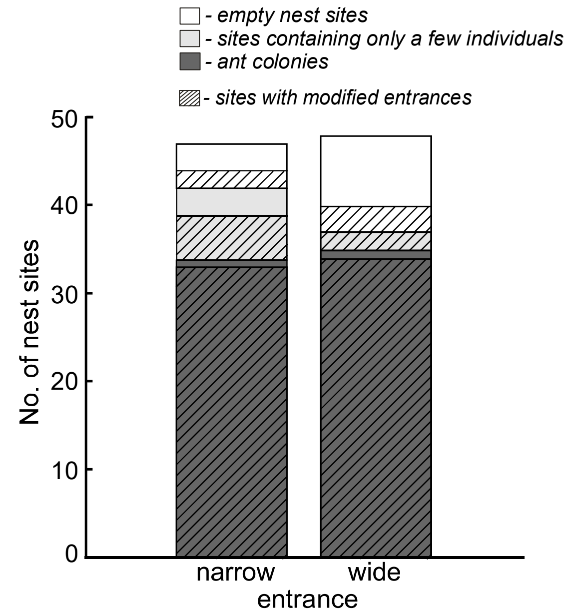

Fig. 3: Number of artificial nest sites with "narrow" and "wide" entrances in which *Temnothorax crassispinus* ant colonies, a few individuals without brood, or no ants, were found and included in analysis. Additionally, the number of artificial nest sites with entrances reduced by sand and soil is shown by shaded areas. As part of this field experiment, 48 artificial nest sites with "narrow", and 48 with "wide" (cf. Fig. 1) entrances were left in April 2018 and collected about three and a half months later.

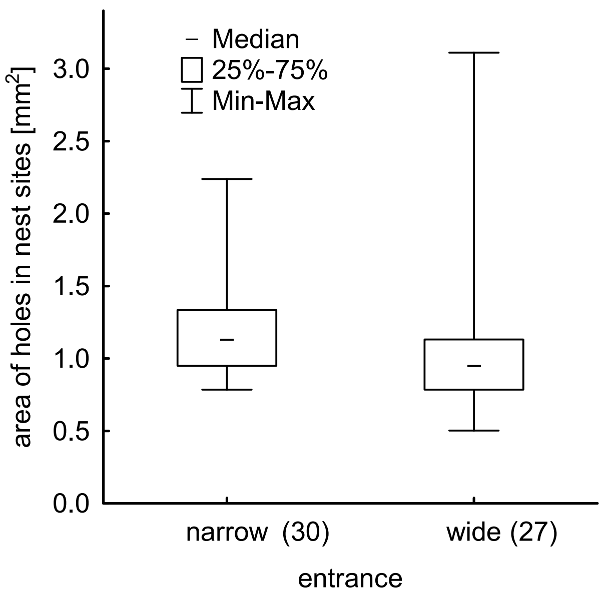

Fig. 4: Areas of the artificial nest cavity entrances after reduction by *Temnothorax crassispinus* ant colonies; the initial areas of the "narrow" and "wide" entrances were about 6.3 and 12.6 mm², respectively (cf. Fig. 1). The numbers of measured holes are given in parentheses.

The entrance was modified (i.e., size was reduced) for the majority of nest sites, using sand and soil (Fig 3; see

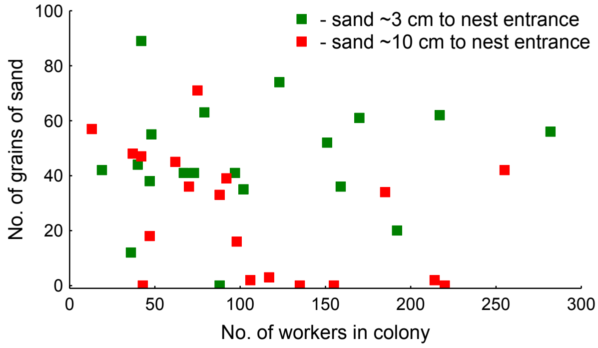

Fig. 5: Number of grains of sand used for entrance reduction in relation to distance of sand from the entrance and number of workers in *Temnothorax crassispinus* ant colonies. During laboratory experiment 1.5 ml of dry sand was deposited about 3 cm, or – for the second group – about 10 cm from entrance to nest. Colonies from the group for which sand was closer to the nest entrances used more grains, but number of workers did not affect the number of used grains.

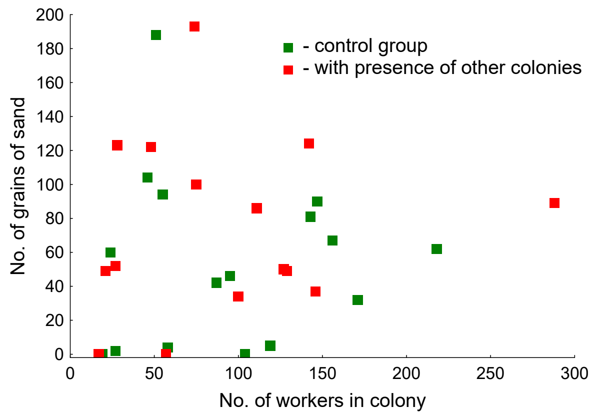

Fig. 6: Number of grains of sand used for entrance reduction in relation to presence of another colony and number of workers in *Temnothorax crassispinus* ant colonies. During laboratory experiment 1.5 ml of dry sand was deposited about 5 cm from the entrance; for the colonies from the experimental group, their Petri dish also contained a nest site with an ant colony from another location (see text for details). There were no differences between the two groups, nor did the number of workers affect the number of grains used.

also Appendix, as digital supplementary material to this article, at the journal's web pages). The structure of sand and soil in the entrance was strong, as though cemented. Most reduced entrances (45 / 61) were round in shape, 12 elliptical, and four irregular (data for nest sites with ant colonies). Areas of the reduced round and elliptical holes were ca. 0.5 - 3.1 mm² (median 1.13, N = 57); the initial areas of the "narrow" and "wide" entrances were about 6.3 and 12.6 mm², respectively. Most modified entrances (39 / 57) had an area of ca. 0.8 - 1.1 mm², and a diameter of 1.0 - 1.2 mm. There was no difference in area of holes in nest sites with "narrow" vs. "wide" entrances (Mann-Whitney U test, Z = 1.50, N = 30, N = 27, p = 0.13; Fig. 4).

Experiment 2 (a laboratory experiment): Does distance between nest site and source of sand affect nest entrance modification? Most colonies (33 / 38) reduced entrances using grains of sand (Fig. 5). The grains were loosely piled at the entrance. As predicted, ant colonies from the group for which sand was closer to the nest entrances used more grains of sand compared to the colonies for which sand was farther (GLM: $F_{1,35} = 7.32$, p = 0.010), but the number of workers did not affect the number of used grains (GLM: $F_{1,35} = 0.72$, p = 0.40).

Experiment 3 (a laboratory experiment): Does presence of other ant colonies affect nest entrance modification? Most colonies (13 / 15 from treatment experimental group, and 14 / 16 from control group) reduced entrance sizes; for the modification they used 4 - 193 grains of sand (Fig. 6). Contrary to prediction, there was no difference in number of grains used by colonies from the treatment group and the control group (GLM: $F_{1,28} = 1.06$, p = 0.31), and the number of sand grains used was not affected by the number of workers (GLM: $F_{1,28} = 0.21$, p = 0.65).

Experiment 4 (a laboratory experiment): Do larger colonies remove more material filling the nest cavity? All 20 colonies in the group with cavities 80% filled with pieces of wood inhabited the cavities. One colony did not remove the pieces of wood; the next six removed only a few pieces. Other colonies removed ca. 0.25 - 1.25 ml of pieces of wood. As predicted, there was a strong positive correlation between the number of workers and the volume of removed pieces of wood (Spearman rank correlation, $r_s = 0.78$, n = 20, p < 0.0001) (Fig. 7A).

In the group for which cavities were 80% filled with dry sand, colonies removed after four days 0 - 105 grains of sand (median 2; nine colonies removed no grains of sand from the cavities; volume of the removed 105 grains was only ca. 0.1 ml). One colony did not inhabit the nest cavity; a portion of workers of two other colonies could not fit in these cavities (colonies containing 256 and 207 workers, which removed 5 and 105 grains of sand, respectively). There was a weak, positive correlation between number of workers and number of grains of sand removed from the nest cavity (Spearman rank correlation, $r_s = 0.52$, n = 19, p = 0.021) (Fig. 7B).

Experiment 5 (a laboratory experiment): Are acorn ants able to dig a nest cavity directly in soil? As predicted, colonies of the acorn ants are able to dig a cavity in the soil: all of the 19 colonies used in the experiment created cavities for nests. Only four of them created cavities under flat stones; others just in sand and soil (see Appendix, as digital supplementary material to this article, at the journal's web pages). One colony created two small cavities: one under stone, the second just in soil. Cavities of seven colonies reached the bottom of Petri dishes (layer of sand and soil was only ca. 10 mm). For two colonies (containing 98 and 214 workers), I was able to measure their nest cavities, both of which reached the bottom of

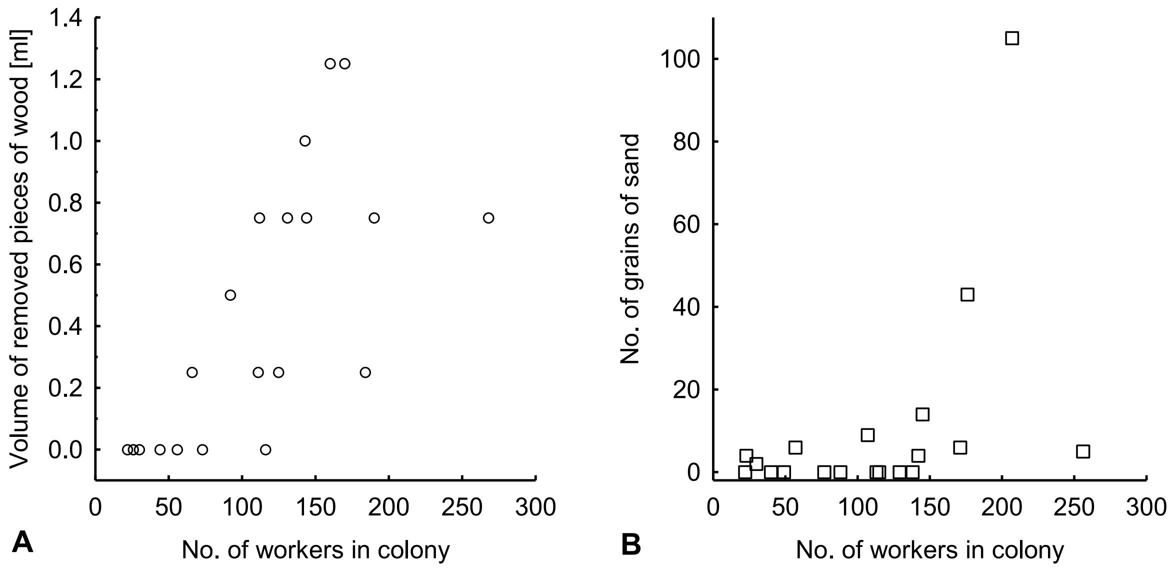

Fig. 7: Correlation between volume of removed pieces of wood (A), and number of grains of sand (B) in relation to the number of workers in *Temnothorax crassispinus* ant colonies. During laboratory experiment, artificial nest cavities (volume ca. 1760 mm³) were filled to ca. 80% of their volume either with small pieces of fresh wood or with dry sand; the material removed by ant workers from cavities was collected after four days. Volume of the removed sand was small; volume of the 105 grains was only ca. 0.1 ml.

the Petri dishes: the bottom of these cavities was round, about 1.5 cm × 1.5 cm.

Discussion

The results of the study show that the acorn ant *Temnothorax crassispinus* can modify available nest sites. If the quality of the cavity was good, but the entrance quite wide, the ants in the field and in the laboratory reduced the size of such an entrance. During the field experiment, I found no difference in proportions of inhabited artificial nest cavities with "narrow" entrances vs. "wide" entrances. This result could be surprising, since – in laboratory experiments – *Temnothorax* have been previously shown to prefer cavities with narrow entrances (e.g., Pratt & Pierce 2001, Franks & al. 2003, Pratt 2010). However, there is a difference between these experiments: During preference tests in the laboratory (cf. Pratt & Pierce 2001, Franks & al. 2003), ant colonies are able to easily compare available nest sites, but during the field experiment, artificial nest sites were not located next to each other. It is known that in the laboratory ants are able to choose superior nest sites, even if these are located at a greater distance than ones of lower quality (Franks & al. 2008). However, in natural conditions good nesting sites are limited (Foitzik & Heinze 1998, Philpott & Foster 2005, Blüthgen & Feldhaar 2009, Jimenez-Soto & Philpott 2015), and competition for such sites could be high (Herbers 1989, Herbers & Banschbach 1995, Foitzik & Heinze 1998). During the field experiment, most artificial nest sites were inhabited by the ants, thus colonies probably compete for such sites. Consequently, the possibility to not adopt optimal nest sites is in such a situation an important ability for ants.

The entrance is a significant part of a nest; for example, it could enable access for predators, and influence microclimate (Halboth & Roces 2017, Lehue & Detrain 2019). I found that *Temnothorax crassispinus* modify entrance sizes: In the field experiment, most artificial nest sites, also with "wide" (worse) entrances, were inhabited by ant colonies, and their entrances were reduced. Further, several nest sites without ant colonies when collected showed entrance modification (see Fig. 3), so the sites were probably earlier inhabited by ant colonies. The diameter of such modified entrances was typically reduced to about 1.0 - 1.2 mm; similar behaviour and similar reduced entrance sizes were reported for *T. longispinosus* (Roger, 1863) inhabiting artificial nest sites made of woodblock (Herbers & Banschbach 1995), and *T. curvispinosus* (Mayr, 1866) in acorns (Pratt & Pierce 2001). Aleksiev & al. (2007a) showed that *T. albipennis* individuals collected more grains of sand to build protective walls in cavities, at 3 cm compared with 40 or 80 cm from nests. Also, in laboratory experiments performed during the study, the ant colonies reduced the sizes of nest entrances. The number of grains of sand used for such modifications did not correlate with colony size, but – similarly to Aleksiev & al. (2007a) results mentioned above – if the source of sand was located farther away, a smaller number of grains was used. Thus, although mean foraging distance of the ant under natural conditions is more than 50 cm (Fokuhl & al. 2012), even several centimetres could influence the volume of transported sand. Nevertheless, in forest litter, sand and soil are not limiting resources, thus workers can easily collect such material near a nest cavity and reduce the entrance size, if necessary. The entrance modifications in the field experiment and laboratory experiments were different. The structure of sand and soil used for entrance modification in the field experiment, was strong, as though cemented. Contrary to this, in the laboratory experiments, grains of sand used for the entrance size modification were put loosely. The difference could result from ants in the field using soil in addition to sand: During the laboratory Experiments 2 - 4, grains of sand but not soil were available to ants. In the laboratory experiments, ants always used a mix of different sized grains; such mix of big and small grains of sand increases the stability of grain piles (Aleksiev & al. 2007c).

The presence of other colonies in Petri dishes did not affect entrance size reduction, but in the experiment the other colonies were not able to leave the nest cavity. Thus, no direct contact between workers from different colonies was possible. Additionally, to ensure that two colonies placed in one Petri dish were not closely related, I used colonies from two locations. However, based on the "dear enemy" hypothesis, a colony has more to fear from its neighbours, with whom it competes for example for food, than from strangers (cf. Gordon 1989). Thus, using two from different locations, could affect the result of the experiment.

The acorn ant *Temnothorax crassispinus* uses sand and soil to reduce nest entrance sizes, and if a suitable cavity is partially filled, they remove pieces of dead wood, but rather not grains of sand. Acorn ants living in forest habitats prefer bigger cavities (data for *T. crassispinus*, see Mitrus 2015), and are able to enlarge cavities (*T. nylanderi*, see Foitzik & Heinze 1998); however, cavity volume is weakly correlated with the size of the occupying colony (*T. curvispinosus*, see Pratt & Pierce 2001). Species which inhabit cavities under stones can remove grains of sand to enlarge cavities (cf. Minter & al. 2013). During laboratory experiments *T. crassispinus* workers typically removed only a few grains from a cavity partially filled with sand, even if available space was too small for the whole colony. I used dry sand in the experiments, but for ants the humidity of soil could affect digging effort (Espinoza & Santamarina 2010, Pielstrom & Roces 2014); however, during another experiment performed in spring 2019, they less frequently inhabited cavities with wet sand (0.3 ml of water per 1.5 ml of dry sand), but if inhabiting such cavities, there was no difference in the number of removed grains from cavities filled with dry vs. wet sand (S. Mitrus, unpubl.). Thus, the result of the study is not simply a consequence of using dry, not wet, sand. As the most recent common ancestor of *Temnothorax* ants was ground-nesting (Prebus 2017), it could be interesting to check whether different groups (i.e., *Temnothorax* ants that typically dwell in cavities in acorns and sticks vs. those living under stones) present different behaviour with respect to using sand / soil and pieces of dead wood.

This study showed also that colonies of the ant *Temnothorax crassispinus* can create cavities for nests under stones or just in soil. Typically, nest density of cavity nesting ant colonies is estimated by opening all potential nest sites, like twigs and acorns (e.g., Strätz & Heinze 2004, Białas & al. 2011). Lörinczi (2011) collected colonies in the top several centimetres of soil surface: two of 56 nest sites with *T. crassispinus* were found in the soil under stones (G. Lörinczi, pers. comm.). Based on results of this study, colonies build nests in soil if there are no available cavities. Such nest sites in soil probably are worse for the ants, as nests in acorns or twigs are potentially safer, and regulation of microclimate, e.g., relative humidity, should be more difficult for ground nests. It would be interesting to compare such "soil" nest sites with typical nest sites for the ants (i.e., in twigs and acorns) in relation to productivity, etc. However, depth of such "soil" cavities could also be important for ant colonies. The layer of sand and soil used during the study was only 10 mm, and in such situations, colonies reached the bottom of Petri dishes. Mitrus (2013) showed that the survival rate of *T. crassispinus* workers in artificial nests experimentally buried at a depth of 5 cm was higher than those in nests on the surface of the soil. During strong frost and lack of snow cover, the survival rate in soil could be higher (cf. Mitrus 2013, 2016). Thus, under natural conditions, the depth of such cavities could be important. Such "soil" nests, probably less safe for workers and brood during spring and summer, may provide better isolation and thus higher survivorship during winter periods.

Many species of social insects, including ants, are a group that considerably affects the environment; they are often described as "ecological engineers" (Jones & al. 1994, Leite & al. 2018). As described in the study, the nest modifications by the *Temnothorax* ants are not large. However, the ability to change nest entrance size and nest volume is important for the ant's ecology. Additionally, because of their large densities, *Temnothorax* ants could influence soil distribution and thus whole ecosystems. For example, they utilize elaiosomes of different plants, e.g., *Chelidonium majus*, *Senecio vulgaris*, *Taraxacum officinale*, and *Viola riviniana*; elaiosomes are fleshy structures consisting of sugar, lipids, and proteins, attached to the seeds. Workers can carry seeds of these plants to their nests to feed the elaiosome to their larvae (Fokuhl & al. 2012), and for seeds it could be favourable if they are deposited in a shallow cavity in soil. Thus, information about using cavities in soil by *Temnothorax*, which typically dwell in cavities in acorns and twigs, could help to understand the structure of ant communities and the influence of ants on soil and ecosystems.

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