

PECULIAR POLYDOMY IN WOOD ANTS (HYMENOPTERA: FORMICIDAE): AN AUXILIARY NEST SEVEN METRES UNDERGROUND

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Abstract.— In this paper we show the unexpected fate of the odd *Formica polycтена* ‘colony’, spontaneously created at the bottom of a concrete post-nuclear bunker by workers falling into it en masse – with no way to leave – from their maternal nest located at the outlet of the bunker ventilation pipe (see Czechowski *et al.* 2016, Rutkowski *et al.* 2019). At the end of previous research, we inserted a wooden structure into the pipe to allow the ants trapped below to return to their maternal colony, which they did. Then it turned out that the ants, now able to move freely to and from the bunker, began to use the lower nest (in the form of an earthen mound) as an auxiliary nest for the colony, mainly as a winter nest (during the research period they inhabited it for two consecutive winters), and the bunker itself as a place for storing corpses and waste. We discussed these findings in the context of seasonal changes in temperature and relative humidity measured for the interior of the lower and upper nest mound and the air inside and outside the bunker. Our results highlight the extreme nesting flexibility shown by this species under very unusual environmental circumstances.



Key words.— adaptability, colony organisation, flexibility of nesting, *Formica polycтена*, extreme habitat conditions

INTRODUCTION

Polydomy, i.e. the creation and use of several spatially separated but socially connected branch nests, is a common social organisation in many ant species (Debout *et al.* 2007). It has developed in a spectacular form in highly polygynous ants, among others, wood ants of the subgenus *Formica* s. str., such as *Formica polyctena* Först. or *F. aquilonia* Yarr. (see e.g. Dlusskij 1967, Zakharov 1968, 2015, Ellis and Robinson 2014, Seifert 2018). A polydomous colony is formed and grows through budding, i.e. spontaneous separation of groups of workers (possibly with brood and some queens) from maternal colonies, which establish branch nests nearby (Bourke and Franks 1995). The functioning of such a complex system as the polydomous colony of ants is possible due to the functional specialisation of individual units (nests) of the colony while maintaining the integration of the entire system. In wood ants, the social connection between nests consists of two-way trails of workers between the nests of the multi-nest colony. Along these trails, colony mates, both adults (including queens) and offspring are exchanged, and food is distributed (Ellis and Robinson 2016).

The concepts and terminology of polydomy, which have grown over the decades, are confusing and tangled (see Debout *et al.* 2007). However, without the special risk of creating even greater chaos, the main kinds of nests within polydomous *Formica* ant colonies, particularly those of the subgenus *Formica* s. str. (i.e. wood ants), can be most generally divided into permanent nests (maternal and filial), seasonal nests (summer and winter) and auxiliary, more or less temporary nests (e.g. foraging stations) [acc. to Forel (1921–1923), Oekland (1934), Marikovskij (1962), Kneitz (1964), Zakharov (1968, 2015), Robinson 2014, Ellis and Robinson (2015) and the authors' observations; compiled].

The possibility of the formation and development of a polydomous system and the spatial arrangement of nests within it are determined by environmental conditions. However, the availability of nest loci plays an important role (Rosengren and Pamilo 1983); a crucial factor limiting this availability may be local thermal conditions (Ellis and Robinson 2014), which, first of all, strongly determine the possibility and rate of brood development (Huey and Berrigan 2001). Therefore, it can be assumed that the choice of the nesting site in wood ants is the result of a compromise between the need for high sunlight, which determines the development of ants' brood, and the need for effective feeding of workers, which is possible rather in fairly shaded places (Ellis and Robinson 2014). On the other hand, mature colonies of wood ants, as

poikilotherms, are strongly dependent on the ambient temperature, which is subject to significant daily and seasonal fluctuations. Such variability in thermal conditions does not support efficient larval development (Huey and Berrigan 2001). Thus, like other social insects (Jones and Oldroyd 2006), ants have developed several thermoregulatory strategies that vary depending on the type of nest (in soil or wood, with or without a mound) and the type of nest/mound material (mineral soil, plant debris), and other factors (see Kadochová and Frouz 2014). For example, the temperature of subterranean nests correlates with soil temperature, so workers move the brood up and down the nest into the soil along a temperature gradient that corresponds to the daily temperature variation in each season (Roces and Núñez 1989, Penick and Tschinkel 2008). The interior of nest mounds made of dry plant material (as, for example, in wood ants) is characterized by relative temperature stability – the larger the colony size (and therefore the larger the nest), the greater the stability. The location of nests and the shape of their mounds are designed to absorb solar energy, and their thermal conditions are also regulated by the ants' metabolism and the decomposition processes of organic matter (Kadochová and Frouz 2014). Moreover, mature, large colonies of wood ants – constantly rebuilding their nests – can actively thermoregulate them (raising and lowering the temperature depending on needs and external conditions). Endogenous processes based on ants' social behaviour (like clustering), as well as the metabolism of the ants also contribute to this (e.g. Martin 1980, Rosengren *et al.* 1987, Stockan and Robinson 2016, Stukalyuk *et al.* 2020).

In addition to temperature, relative humidity plays an essential role in the establishment and survival of ant colonies (Mankowski *et al.* 2011). It also influences the occurrence of ants infected by entomopathogenic fungi (Neto *et al.* 2019) and affects the deposition of the waste within the colony (Römer *et al.* 2019). Moreover, environmental moisture significantly impacts individual performance and survival during foraging (Menzel *et al.* 2018, Azhar *et al.* 2024), leading to upregulation of physiological adaptations against desiccation (Menzel *et al.* 2018). For example, *Formica rufa* workers are slightly more permeable to water than males and queens, and callow workers lose water at a higher rate than mature workers, which explains their preference for more humid environments (North 1991). Furthermore, it is the photoperiod, rather than seasonal effects, that primarily influences the humidity preferences of *F. rufa* workers (North 1991). In red wood ants, however, the mound's moisture content has a direct effect also on the nest's thermoregulation efficiency (Frouz 2000). A humid surface prevents the penetration of cooling

winds into the nest but results in higher thermal loss and increased microbial heat. Conversely, a dry surface confers different isolation properties and leads to greater thermal fluctuations within the nest (Frouz 2000, Sorvari *et al.* 2016).

In addition to the above-mentioned thermal strategies, ants owe their evolutionary and ecological success achieved in all terrestrial environments to their extraordinary behavioural plasticity, manifested by the ability to use new, often artificial resources to achieve their thermal goals even in extreme conditions. For example, we can mention the use of heat from ground bird nests by species of the genus *Myrmica* Latr. (Maziarz *et al.* 2021), the use of slate plates as artificial nesting sites by several ant species (Gallé *et al.* 2014), using the bottom of a broken large glass jar discarded in the forest as an incubator for pupae by *Formica sanguinea* Latr. (W. Czechowski, unpubl.) or nesting of *Lasius niger* (L.) under polystyrene foam insulation, using the hook hole as a nest entrance on the eighth floor of a large-panel building (W. Czechowski, unpubl.).

The subject of the present paper is the case of *F. polyctena* colony and its odd filial nest – odd due to its peculiar origin, history and extremely unfavourable, in seemingly every respect, location: deep underground at the bottom of a concrete bunker (see Czechowski *et al.* 2016, Rutkowski *et al.* 2019; see also Material and methods). The aim of our research was (1) to reveal the further fate of this, unusual in every respect, auxiliary nest, (2) to answer the question of

whether and how ants could benefit from such an unfortunate nest location, and (3) determine possible importance of this nest as a component of the polydomous wood ant system for the colony, and therefore the reasons for its – undoubtedly energetically expensive – maintenance by the colony.

MATERIAL AND METHODS

The colonies

The observations were made at Templewo (52°27'N. 15°23'E) near Międzyrzecz, W Poland in the years 2017–2019. There, close to the German border, a Soviet nuclear base existed from the late 1960s to 1992. Currently, the remaining part of the former military complex are two abandoned underground two-story ammunition bunkers with an extensive system of rooms and corridors covered with spruce forest. Directly above one of the bunkers – on a hill created by covering it with a layer of soil – there are three large (matured) nests of *F. polyctena*, which are part of a polydomous system composed of a total of seven mature nests (Table 1).

Further down from bunker hill lies the remaining part of this multi-nest colony, with the nearest nests approximately 50 meters apart. This section consists of four large nests and two small auxiliary nests (probably foraging stations) (Fig. 1a). The core of the mound of one of the colonies above the bunker is

Table 1. The size (mound volumes; dm³) of the *F. polyctena* nests from the bunker polydomous system and two other nearby (within a radius of 1.5 km) polydomous systems (A and B) measured along a season between April 26–27, 2018 and September 23–24, 2018. Within the bunker system, Nests 2 and 3 were situated on the top of the bunker hill, in close connection with the over-bunker-nest, whereas the other nests were down the bunker hill, situated further from these colonies, being in a looser contact with them (small incipient or auxiliary nests are Pmarked with an asterix).

Bunker-system			System B		System C	
Nest	Date		Nest	Date	Nest	Date
	04.2018	09.2018				
Bunker-nest	131.37	226.83	Nest 1	146.53	Nest 1	2316.49
Over-bunker-nest	954.51	1802.97	Nest 2*	2.89	Nest 2	47.31
Nest 2	643.74	1686.42	Nest 3	18.33	Nest 3	118.96
Nest 3	623.28	638.21	Nest 4*	11.45	Nest 4	60.09
Nest 4	239.98	329.78	Nest 5	34.02	Nest 5*	16.79
Nest 5*	96.74	69.77	Nest 6*	13.96		
Nest 6	115.16	153.98	Nest 7	67.17		
Nest 7*	30.73	67.05	Nest 8	19.07		
Nest 8	115.15	245.29	Nest 9*	5.22		
Nest 9	258.64	238.34	Nest 10	134.26		
			Nest 11*	6.39		
			Nest 12	24.66		
			Nest 13*	12.99		

located at the very outlet of the bunker's ventilation pipe (see Figs 4 and 5 in Czechowski *et al.* 2016). Therefore, ants from this nest were constantly accidentally falling down this pipe straight from the nest into one of the rooms of the bunker. Over the years, living mostly by cannibalism on their dead (see Rutkowski *et al.* 2019), the bottom 'colony' has been growing due to the constant influx of individuals falling from the maternal nest; in 2015, the size of the colony was estimated at several thousand alive workers. Moreover, apart from the polydomous system mentioned above, within a 1.5 km radius there were two other *F. polycтена* polydomous systems: the larger one (13 nests; hereafter the system B; Fig. 1b), located 1384 m in a straight line from the closest nest of the bunker system, and the smaller one (5 nests; hereafter the system C; Fig. 1c), located 995 m from the nearest bunker system nest.

Here we present new observations and research conducted in the years 2017–2019, i.e. after the experimental installation of a wooden structure ('ladder' in the bunker in autumn 2016, which allowed the ants trapped in the bunker to return to their maternal nest

(see Rutkowski *et al.* 2019), while giving them the opportunity to move freely between the 'upper' and 'lower' nests. Our particular goal was to determine the role of the bunker nest in the life of the over-bunker colony at that time.

Field works

We collected data for this study during five two-day trips to the bunker and its surroundings on December 5–6, 2017, January 20–21, April 26–27, August 23–24, 2018 and May 29–30, 2019. The location of all *F. polycтена* nests belonging to the bunker-nest polydomous system, i.e. the nest inside the bunker (hereafter referred to as 'bunker-nest') and its maternal nest (hereafter referred to as 'over-bunker-nest') and all other nests of this polydomous system, as well as those of two other polydomous systems were marked out using GPS (Garmin Oregon 700).

We measured the mound of each nest, taking into account the height and two perpendicular diameters. Based on these dimensions, we determined the volume (V) of each mound (earth one in the bunker-



Figure 1. The situation of the three discussed polydomous nest systems of *F. polycтена* and the distribution of nests within each of them. (a) The bunker nest system (pink dots – nests on the top of the bunker hill; purple dots – nests below the bunker hill); (b) The nest system B (red dots; 1384 m east of the nearest nest of the bunker nest system); (c) The nest system C (yellow dots; 995 m east of the nearest nest of the bunker nest system). The maps were generated in QGIS 3.36.0 (QGIS 2024).

nest) according to the formula for the volume of a semi-ellipsoid:

$$V = 2/3 * \pi * r_1 * r_2 * h,$$

where h is the height of the mound and r_1 and r_2 are two perpendicular radii of the mound's base. The volume of an above-ground mound is used as a good measure of the relative colony size of wood ant colonies (Risch *et al.* 2005, Czechowski and Vepsäläinen 2009, Puntila and Kilpeläinen 2009). We performed measurements of the nest mounds of the bunker polydomous system in each of the five research periods; moreover, once in 2018, we measured the mounds of each of the two neighbouring polydomous systems: in April (system B) and in September (system C).

On the five dates given above, we recorded the temperature and relative humidity of the nest mound and its immediate vicinity for the bunker-nest and the over-bunker-nest. Measurements were taken every five minutes for at least 19 hours using Voltcraft DL-121TH data loggers. The device's sensors were embedded in the soil of the nest mound of the bunker-nest and the different holes in the wall in its vicinity, whereas in a depth of 15 cm in the over-bunker-nest mound, and suspended 3–5 cm above the ground in the vicinity of the over-bunker-nest.

To assess the possible difference in the physical condition of the workers staying in the bunker-nest and those from the over-bunker-nest, on April 27, 2018, we collected 101 workers from the bunker-nest and the over-bunker-nest, respectively, and weighed each of them to determine their fresh matter on an analytical precision balance Ohaus Explorer Pro EP214 (0.1 mg accuracy).

Statistical analyses

The weight of the workers from the bunker-nest and the over-bunker-nest were compared using a GLM model (Gaussian error, maximum likelihood). In the model, the weight of the workers was included as dependent variable, whereas their origin (bunker-nest vs. over-bunker-nest) as explanatory factor.

The statistical analysis was carried out in the R Statistical Environment (R Core Team 2022). GLMs were performed with the `glm` function. Variables were log-transformed for normalisation.

We used QGIS 3.36.0 for distance calculations and the visual representation of our data (QGIS 2024). A shape file (SHP) was generated based on the GPS location of each nest. Using this vector file, we calculated the nearest neighbour index and z-scores of the studied sites using the Nearest Neighbor Analysis tool.

RESULTS

The polydomous system of *F. polyctena*, which included the bunker-nest discussed here and its maternal nest (i.e. the over-bunker-nest), had a total of 10 nests. Three of them (including the over-bunker-nest), all inhabited by mature colonies, were located on the hill directly above the bunker, and the rest (four large and two auxiliary) at some distance, below the hill. In addition, there were two other *F. polyctena* polydomous systems (B and C) in the searched area, numbering 13 and five nests, respectively (Fig. 1, Table 1).

In May 2019, the nest size of the over-bunker nest was 2291.86 dm³, whereas that of the bunker-nest was 257.06 dm³. During one year, similarly to the increase in the over-bunker-nest (1013.14 dm³ increase), the bunker-nest also almost doubled its size by showing a 95.21 dm³ increase (Table 1). Moreover, the weight of the workers measured at the end of spring was significantly larger in the over-bunker-nest compared to the bunker-nest (8.8 ± 3.6 mg vs. 5.4 ± 1.9 mg, mean \pm SD; $z = -8.38$, $p < 0.001$; Fig 2). On the other hand, one of the two other nests on the top of the bunker, i.e. Nest 2 (having a strong internest connection with the over-bunker nest) showed a similar increase along the season: 789.9 dm³; Table 1). The over-bunker-nest and Nest 2 were located at the bases of foraging trees (diameters: in the over-bunker nest – 59 cm; in Nest 2 – 91 cm), but they were tending aphids also on other trees in the vicinity.

The remaining nests of the bunker-system (below the bunker hill; Fig. 1a) also showed an average increase (excluding the two auxiliary nests that decreased) of 45.17 dm³ along a season, although their increase was not so striking (Table 1). In comparison, the close-by nest system B showed a large variability containing several incipient nests (smallest 2.19 dm³, largest 111 dm³). The average nest size (\pm SD) within this system was 28.96 ± 36.63 dm³ (Fig. 1b; Table 1). On the other hand, nest system C containing five nests (smallest 12.73 dm³, largest 1754.9 dm³; Fig. 1c; Table S1), had an average (\pm SD) nest size of 387.82 ± 764.74 dm³. The nests of these polydomous systems were mainly formed on fallen trees or tree trunks left behind after cutting, showing a more dispersed nest location governed by random processes, as supported also by the nearest neighbour analysis of the bunker-system (NNI = 1.42, $z = 2.42$; only the nests below the bunker: NNI = 1.76, $z = 3.57$; Fig. 1a), the nest system B (NNI = 1.31, $z = 2.12$; Fig. 1b) and the nest system C (NNI = 2.08, $z = 4.62$; Fig. 1c). This was even more outlined if we considered only the three nests on the top of the bunker (NNI = 3.26, $z = 7.48$).

During our two first winter visits to the bunker after resuming research (i.e. December 2017 and January 2018), we found the bunker-nest in the form of an orderly (regularly shaped) earth mound (with a slight admixture of spruce needles), and fully inhabited by ants – in contrast to the situation from the previous winter, i.e. shortly after the ‘ladder’ was inserted allowing the trapped ants to return to their maternal nest (Fig. 3; cf. Fig. 5 in Rutkowski *et al.* 2019). Unlike the colony in the still snow-covered over-bunker-nest, the ants in the bunker-nest were already fairly active (Supplementary online material), though there was no movement on the ‘ladder’ connecting the lower nest with the upper one (Fig. 3).

In the periods from spring to autumn in the following years (2018 and 2019), we observed a significantly increased number and activity of ants in the bunker, as well as an intensive exchange of workers between both nests – the over-bunker-nest and the bunker-nest (Figs 4 and 5). Despite several careful inspections of the interior of the bunker mound, we did not find larvae, pupae or queens; although we revealed clusters of hibernating, semi-numb workers under pieces of rubble contained in the earth mound.

Already during the first research period, i.e. at the turn of autumn and winter (5–6 December 2017), the bunker-nest turned out to be inhabited by ants (Fig. 3), showing that part of the *F. polyctena* colony from the over-bunker-nest was already voluntarily choosing this place for wintering. The mean air temperature in the bunker was 4.52°C higher than the outside temperature and 3.29°C higher in the bunker-nest than inside the over-bunker nest (Fig. 6). These tem-

perature values also show that in this period inside the bunker-nest it was on average 0.33°C warmer than outside the nest, and inside the mound of the over-bunker-nest it was 0.90°C warmer than outside (Fig. 6). The distribution of relative humidity (Rh) values in early December followed a different pattern. The average humidity in the bunker-nest was fairly higher (by 3.32%) than the air humidity in its surroundings ($91.87 \pm 1.05\%$). However, this difference was noticeably larger than the difference (0.41%) in analogous values for the over-bunker-nest (Fig. 6).

In the middle of winter (20–21 January 2018), the activity of ants from the bunker-nest, due to lower temperatures, was reduced, although it remained above zero and many ants walked on the surface of the mound. There was no extra-nest activity of ants from the snow-covered over-bunker-nest. The average air temperature was 7.65°C warmer inside the bunker than outside. In the mound of the over-bunker-nest, the average temperature was 1.29°C warmer than the air temperature, while in the bunker-nest it was only 0.14°C lower than outside the nest (Fig. 6). However, the relative air humidity in the bunker was 8.19% lower than outside. In turn, within the mound of the bunker-nest, the average humidity was 4.1% higher than the air humidity in the bunker, while in the mound of the over-bunker-nest the humidity was 2.78% higher than in the ambient air (Fig. 6).

Next spring (26–27 April 2018), there were incomparably fewer ants in the bunker-nest than in previous periods. There were also fewer ants on the ‘ladder’, including those carrying workers in both directions and carrying debris (e.g. empty cocoons) down. These items ended up in rubbish dumps or ‘cemeteries’ located mainly under the bunker walls (Figs 7 and 8). The average air temperature outside the bunker was 3.31°C higher than inside the bunker. The average temperature inside the bunker-nest was slightly higher (by 0.07°C), and the temperature inside the mound of the over-bunker-nest much higher (by 10.48°C) than the temperature of their surroundings (Fig. 6). The relative humidity of the air outside the bunker was on average 7.93% lower than inside. The humidity of the bunker- and over-bunker nests was quite similar to each other (only 0.57% higher in the over-bunker nest; Fig. 6).

At the turn of summer and autumn (23–24 September 2018), there was a large number of ants present in the bunker-nest and several dozen workers on the ‘ladder’. Some of them carried their nestmates down (adult transport), as well as particles of nest material and food items (dead insects, including beetles). The average air temperature inside the bunker was 1.51°C higher than outside the bunker. On the other hand, the average temperature in the mound of the bunker-nest was 8.91°C lower than in the mound

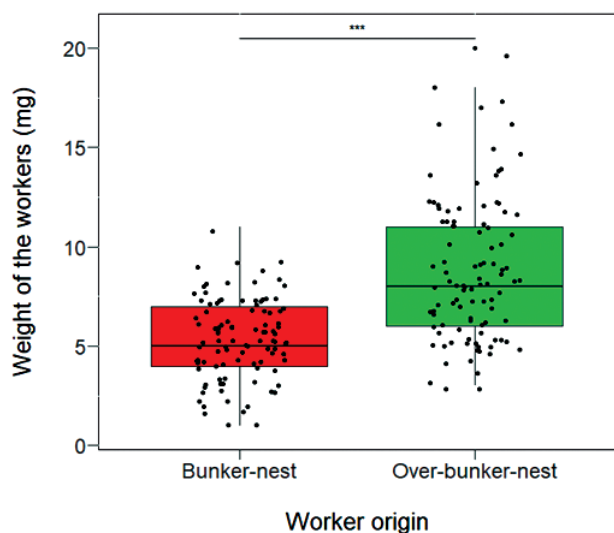
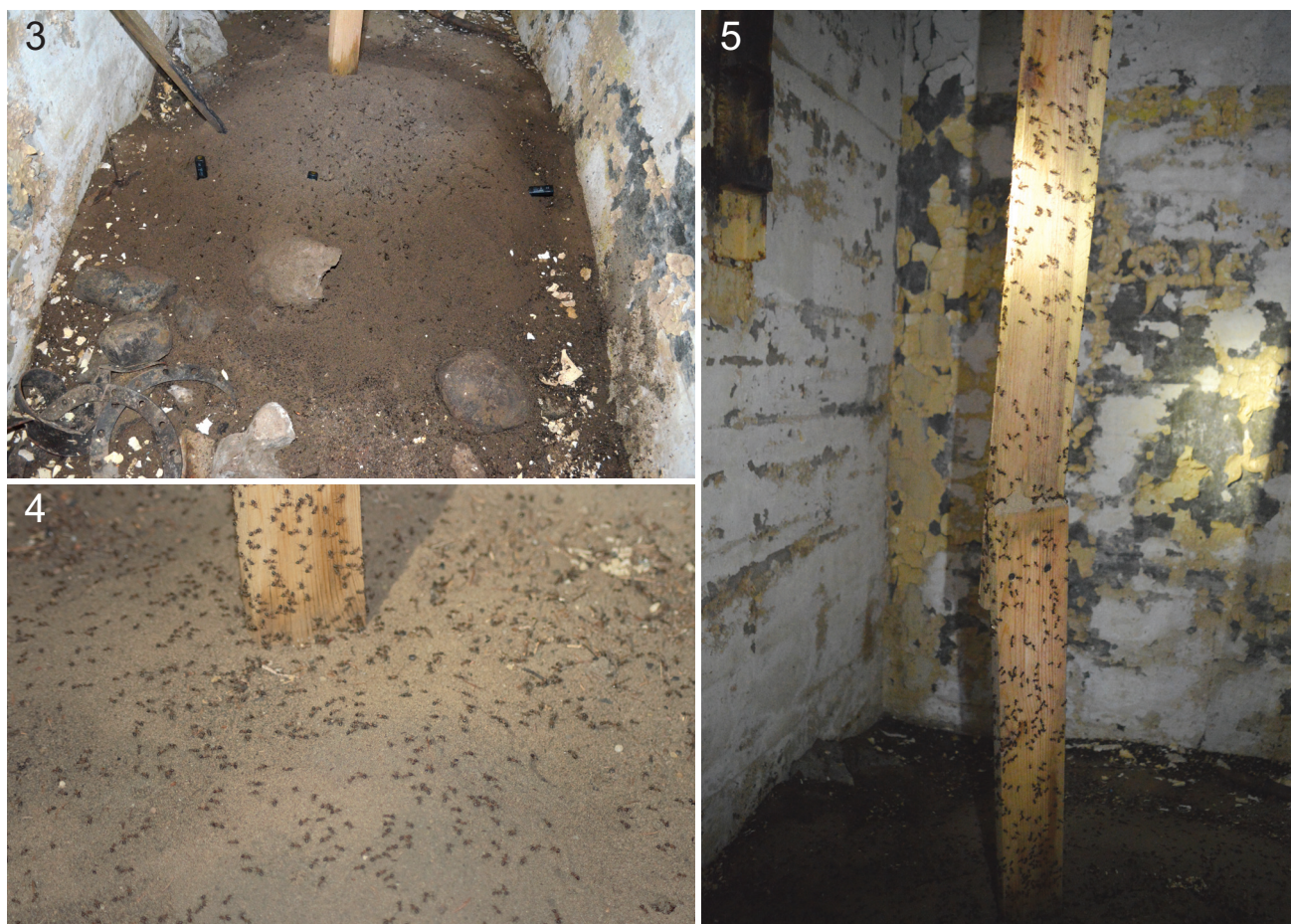


Figure 2. Differentiation of body weight of *F. polyctena* workers from the bunker-nest (red) and the over-bunker-nest (green) in April 2018 (N = 101 in both cases).



Figures 3–5. (3) The appearance of the earthen mound of the bunker-nest of *F. polystena* in January 2018. Visible is lower part of the 'ladder' leading to the inlet of ventilation pipe in the ceiling and sensors of the temperature and humidity loggers; (4) Activity of the *F. polystena* ants on the mound of the bunker-nest and on the lower part of the 'ladder' in May 2019 (cf. Fig. 3); (5) Exchange intensity of *F. polystena* workers between the bunker-nest and the over-bunker-nest in May 2019 (Photos by I. Maák and G. Trigos-Peral

of the over-bunker nest (Fig. 6). The relative air humidity values inside and outside the bunker were very similar: 0.28% higher in the bunker. However, the nest mounds differed greatly in terms of their humidity, with the humidity registered in the bunker-nest being 26.27% higher than in the over-bunker nest (Fig. 6).

Next spring (29–30 May 2019), there were again a few ants in the bunker-nest, and the few workers present on the 'ladder' carried the nestmates upstairs and the debris downstairs. We also managed to notice a live, fully fit male on the surface of the mound (Fig. 9) – undoubtedly an accidental newcomer from the over-bunker-nest. The average air temperature inside the bunker was 3.69°C colder than outside the bunker. An even greater temperature difference concerned the interiors of the compared nests: in the mound of the bunker-nest it was on average 7.18°C colder than in the over-bunker nest (Fig. 6). The average air humidity was 14.66% higher in the bunker,

and also the humidity within the mound of the bunker-nest was 16.53% higher than in the mound of the over-bunker nest (Fig. 6).

DISCUSSION

The ecological evolution of species is expressed in their tendency to increasingly control and use the environment (Allee *et al.* 1949). Wood ants, and especially those of the *F. rufa* complex are a spectacular example of animals that have achieved undoubted success in this field. In their typical habitats, i.e. mainly North Palearctic coniferous forests, thanks to their great abundance and biotic pressure on the environment, wood ants have gained a dominant position not only within multi-species ant communities (see e.g. Vepsäläinen and Pisarski 1982, Savolainen and Vepsäläinen 1988, 1989, Pisarski and Vepsäläinen 1989), but also in entire forest zoocenoses. The reasons for

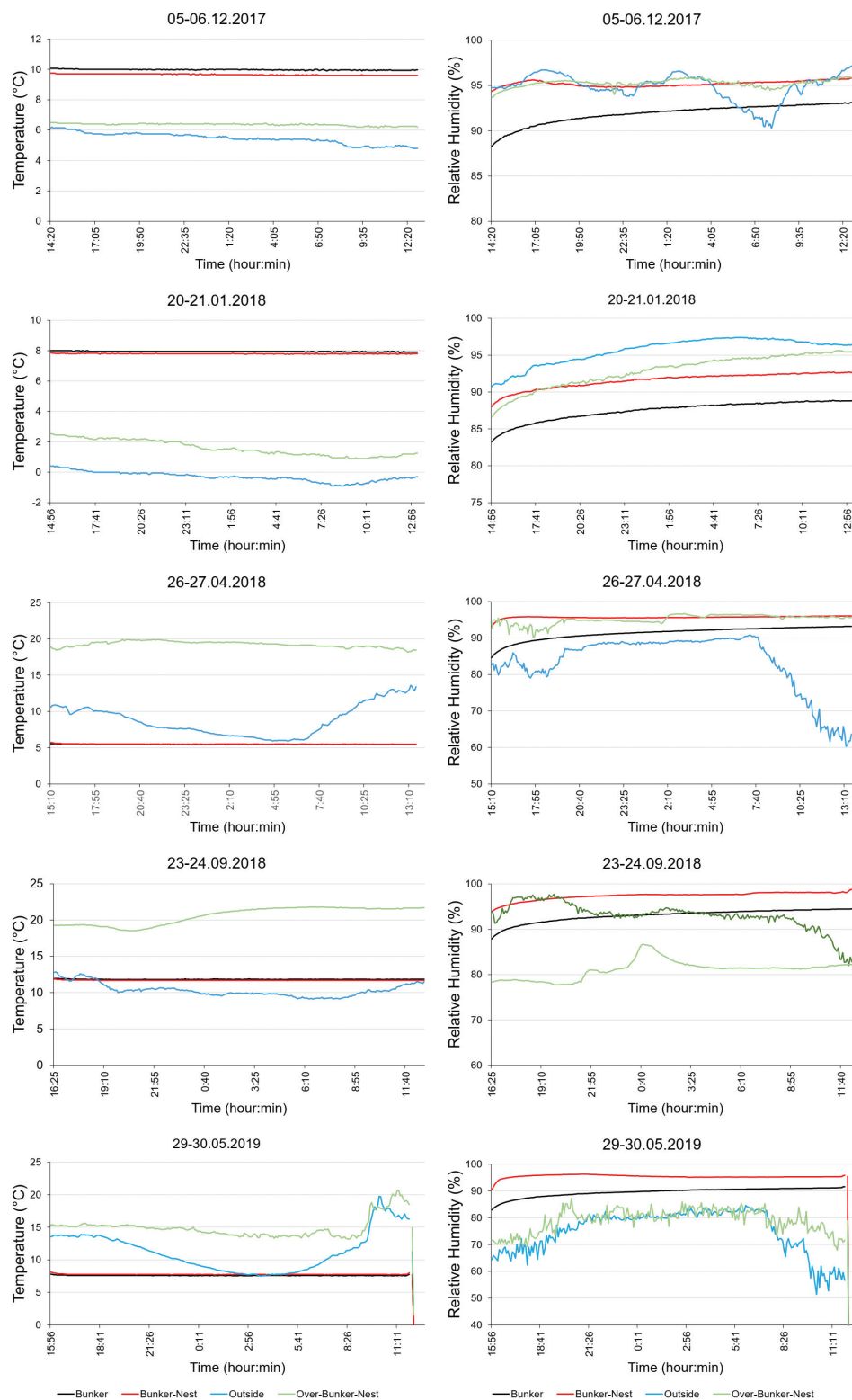


Figure 6. Changes in temperature (left column) and relative humidity (right column) inside the mound and the surrounding air for the bunker-nest and over-bunker-nest.



Figures 7–10. (7 and 8) Rubbish dumps (ant ‘cemeteries’) of *F. polyctena* in the bunker; (9) A male of *F. polyctena* seen on the bunker-nest mound in May 2019; (10) The accumulation of soil on the ‘ladder’ ledge, most likely resulting from soil falling from the *F. polyctena* over-bunker-nest (Photos by I. Maák).

this should be sought both in the features of the biology of the wood ants themselves (among others, polygyny and polydomy, compound nest construction or territoriality) and in the habitats they settle (stable and trophically rich) (see Pisarski and Czechowski 1994, Czechowski 1996). At the same time, these ants are able to survive in some natural habitats believed to be far from optimal for many years, for example, after experimental transfer to rocky islands and islets, like Finnish skerries (see Czechowski and Vepsäläinen 2009, 2010).

The extraordinary survival capabilities of ants as a society (not as individuals), and their great ability to self-organise even in a seemingly hopeless situation, is clearly demonstrated by the history of ‘bunker ants’

described by us, starting from their dramatic origin (see Czechowski *et al.* 2016, Rutkowski *et al.* 2019). The soil falling from the pipe is evidenced by the accumulation of soil on the ‘ladder’ ledge – where the boards are connected (Fig. 10), already present in January 2018. The formation of such a soil mound on the bunker floor must have taken years. During this time, their only potential food available were the dead bodies of their nestmates (Rutkowski *et al.* 2019) – usually used by these ants only in food shortage periods (see e.g. Maák *et al.* 2020). Once connected, the launch of mutual worker exchange between the bunker-nest and the over-bunker-nest had to – in the very nature of things (trophalaxis) – solve the problem of feeding the ants in the bunker. Therefore, it is

difficult to explain why (at least during the study period) *F. polychtēna* workers from the bunker turned out to be significantly lighter than those from the outdoor nest. The difference resulted rather from the nutritional status of the adults of the compared parties, as it is difficult to believe that under conditions of unrestricted exchange, ants from both groups differed in size, and the possibility of offspring developing in the bunker should rather be ruled out.

Still, larvae (due to their important role in colony growth) can be the indirect reason for our observations due to their role as primary receptors during trophallaxis. For example, Cassill and Tschinkel (1995) showed how the bigger and hungrier larvae of *Solenopsis invicta* were more frequently fed than the other larvae. Moreover, larvae from many species (including *Myrmica rubra* or *Formica exsecta*) can beg for food, becoming the main targets of foragers' trophallaxis (Peignier *et al.* 2019, Creemers *et al.* 2003). On the other hand, the redistribution of resources among the neighbouring nests is organised at the local level and not at the entire polydomous colony level, as the exchange of resources is performed directly from a nest with foraging excess to nests that require resources (Ellis *et al.* 2014). Thus, the over-bunker nest would receive the greatest amount of food gathered by the foragers, therefore ensuring the colony's growth and survival, whereas the bunker-nest would only receive the 'leftovers'. This is even more likely considering the time of weight control – late April. At the turn of winter and spring, when there is still a shortage of food in the environment, sexual larvae appear in nests of mature (like the over-bunker-nest) wood ant colonies of the species of the *F. rufa* complex (Dlusskij 1967). At the turn of April and May (at least in *F. polychtēna*; Seifert 2018) there are already numerous adult sexuals – constantly requiring feeding. A complementary explanation for the lower mass of ants in the bunker is also possible based on the time of this study, when there is a high chance that a significant part of the ants present in the bunker could still be individuals wintering there – lighter after hibernation.

We now turn to the main aim of this study, namely to decipher the role of the bunker-nest as a branch nest of the *F. polychtēna* colony and the possible benefits that its maintenance brought to this colony. Initially (it is not known for how long), the bunker was a death trap for the ants that fell into it. By inserting the 'ladder' into the bunker, the thousands of still living ants had the opportunity to return to the maternal nest. Meanwhile, it turned out that the colony used this structure to expand its territory into the underground zone, and turned the earthen nest at the bottom of the bunker into its auxiliary nest. The bunker zone became a place for ant cemeteries and waste

dumps. In ants (including wood ants), debris (corpses of the nestmates, empty cocoons, remnants of consumed prey) are carried out from the nest and usually piled up in refuse dumps, often on the border of the colony's range (in wood ants, equivalent to the border of the territory) (see Lubicz Niezabitowski 1933, Czechowski 1976, Diez *et al.* 2014) or deposited in special 'burial chambers' in the deepest parts of the nest (Staeger 1926, Moser 1963, Farji-Brener and Medina 2000). So we can say that the 'crypt' seven metres underground met all the conditions for this.

As for the purpose of the bunker-nest itself, inspections carried out at different times of the year showed that it was inhabited in autumn and emptied in spring, so it served primarily as a winter nest. Ants of several species of the temperate zone move to the seasonal (winter) nests, located in places that provide better shelter from frost than this would do a more exposed and shallower summer nest. As it seems, the compound, deep and thermoregulated nests of wood ants of the *F. rufa* complex generally meet the conditions to provide the colonies with all their living needs throughout the year (including wintering); the ants that spend the winter in the underground part of the nest, where the temperature does not drop below 5°C (Dlusskij 1967, Sejma 1998). However, it sometimes happens that these ants also move to another place for hibernation (e.g. Kneitz 1964, for *F. rufa* L.). We assume that this applies, first of all, to the species and colonies that live in habitats that are convenient in summer, but for some reason do not allow for adequate expansion of the underground part of the nest to ensure safe wintering, like e.g. those in rocky habitats. This is confirmed by observations from Finland regarding colonies of *F. polychtēna* nesting on rocks and moving in autumn to places with greater soil thickness (R. Rosengren, pers. comm. to K. Vepsäläinen). The dependence of the colony seasonal cycle on the habitat conditions is well illustrated by the cases of *F. truncorum* (a wood ant species outside the *F. rufa* complex) in Finland and Japan (in Japan, the species known until recently as *F. yessensis* has recently been synonymised with *F. truncorum*; Seifert 2021). In Finland, colonies of *F. truncorum*, which nested in rocky outcrops, moved for wintering (at a distance even up to 100 m) to winter nests in the forest (Rosengren *et al.* 1985, Elias *et al.* 2005) or at least in a nearby depression filled with thicker soil (K. Vepsäläinen, pers. comm. to W. Czechowski). A similar seasonal shift of nest sites between the reproduction and hibernation season in this species was observed in Japan (Ito 1973, Imamura 1974).

The bunker-nest as a winter nest for *F. polychtēna* is an extraordinary situation in every respect, starting with the fact that it does not appear that the *F. polychtēna* colonies over the bunker – given their suitable

environment (coniferous forest) and good nest condition – would require special nests for hibernation. The ants simply took advantage of the opportunity: the potential ‘winter nest’ was located directly beneath the over-bunker-nest, functioning as an extension of the underground part of the latter. It is not difficult to imagine that ants descending to overwinter in the subterranean part of the nest found themselves directly on the way to the bunker-nest. Finding stable and optimal conditions for hibernation there, they remained in the bunker-nest for the entire winter. However, based on the minimal temperature differences between the bunker nest and its surroundings, we can infer that the bunker nest does not support enhanced microbial respiration despite its higher humidity compared to its surroundings (Frouz 2000). This may be attributed to the absence of a suitable microclimate, particularly during winter, the lack of appropriate nesting material, or a combination of both factors. Nonetheless, considering that wintering ant colonies in temperate zones are subject to two contradictory selection pressures (safely sheltering during lethal thermal conditions, and reactivating as quickly as possible when conditions permit; see Włodarczyk 2021), we can conclude that the opportunity to overwinter, at least partially, in the bunker enabled the over-bunker colony to help reach a compromise between these two forces.

Despite that wood ants can regulate the temperature in their nests where their offspring are kept, the effectiveness of thermoregulation depends on external conditions, and these on the season. Although various factors are involved in the thermoregulation of nests, the most important seems to be solar radiation and heat released as a result of the metabolism and respiration of the ants themselves (Frouz and Finer 2007). In summer, these sources are practically unlimited, in winter, however, the sunlight is much lower and the activity of ants ceases – not to mention the drastically reduced external temperature. In spring, the nests begin to self-heat and ant activity increases, allowing the workers to once again provide the colony with the warmth and food needed for the colony to thrive. However, in large nests, this process needs to be accelerated, so the heating of the nest is started by the young workers who use their lipid reserves to release metabolic heat (Martin 1980). Thus, the existence of an axillary nest, which is a special winter nest in the bunker that keeps relatively constant favourable thermal conditions, might help to enhance colony production since it can accelerate the activation of the maternal colony and redirect fat reserves of workers to provide food for queens and offspring instead of heating the nest. Thus, this unusual auxiliary nest (the bunker-nest) can help in the earlier start of the colony and enhance its productivity.

The extraordinary nesting conditions of the over-bunker nest gained after the connection with the bunker-nest led to a fast increase in nest size (that correlates with the colony size and is a good indicator of the colony’s well-being; Sorvari *et al.* 2011, Chen and Robinson 2014) found during the observation period. Thus, it seems that the nesting conditions may fulfill the main criteria for optimal nest site locations, namely optimal thermal conditions, and the need for effective feeding of workers, which is possible in fairly shaded places (Ellis and Robinson 2014). Considering that the foraging trees are present near the over-bunker-nest, and suitable thermal conditions might be available also due to the presence of the bunker-nest, these factors may determine a more efficient food source exploitation and competitive advantage. Moreover, our results also showed that the nests within all studied polydomous systems (bunker-system, system B, and system C) in the area were randomly distributed and strongly interconnected. Given that connection among anthills can facilitate faster growth of the interconnected domes through more efficient food distribution (Stukalyuk and Kozyr 2024), the benefits of the bunker might indirectly extend to the nests within the bunker-system. The earlier activation of the workers belonging to the bunker nest would also hasten the activation of internest connections among the nests of the bunker-system and induce their earlier activity. However, further observations are needed to confirm this hypothesis.

To conclude, this study shows the switching from an accidental fatal event (i.e. falling into the bunker) to an advantageous scenario by using the thermal conditions of the bunker for overwintering. The bunker provides more favorable thermal conditions than its surroundings from approximately September to March, with temperature differences reaching up to 8°C in mid-winter compared to outside conditions. Thus, the bunker-nest, functioning as an axillary nest, maintains relatively constant favourable thermal conditions during winter, which may facilitate an earlier start for the colony and enhance its productivity by accelerating the activation of the maternal colony. This adaptation allows the over-bunker-colony to overcome two opposing selection pressures. Additionally, the findings confirm the nesting flexibility of this wood ant species, enabling it to survive in various environmental conditions and capitalize previously suboptimal conditions.

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Supplementary online media material 1. Recording of the ant activity in the bunker nest in January 2018 (G. Trigos-Peral). Video accessible in <https://figshare.com/s/b88824a9c5cb5f89d0e5?file=46540414>
Doi: 10.6084/m9.figshare.25901881