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Termoregulace lesních mravenců rodu *Formica* na výškovém gradientu
Thermoregulation of Wood Ant Genus *Formica* on Elevation Gradient

DIPLOMA THESIS

Diploma thesis supervisor: Doc. Jan Frouz CSc.

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V Praze, 29.8.2011

Podpis

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ABSTRAKT

Tato studie se zabývá termoregulačním chováním lesních mravenců rodu *Formica* na výškovém gradientu. Dvouleté záznamy z datalogerů byly kombinovány s detailním ručním měřením teploty mraveniště v různých hloubkách pod povrchem. Ruční měření probíhala od dubna do září. Výsledky ukazují, že teplota hnízda byla vyšší než teplota vzduchu ve všech ročních obdobích. Teplota mraveniště je nejvyšší v nejhlubší vrstvě, teplo teče zevnitř ven. Domníváme se, že teplotní stabilita mravenčího hnízda na jaře a v létě, tedy v období nejvyšší mravenčí aktivity, je ovlivňována především vnitřními zdroji tepla – mikrobiální aktivitou a metabolismem mravenců. Oslunění zřejmě hraje přímou roli v zahřívání hnízda pouze brzy na jaře, v létě je vliv oslunění na teplotu hnízda nepřímý, skrze vliv na aktivitu mravenců. V zimě hraje významnou roli velikost hnízda, jež koresponduje s izolačními vlastnostmi hnízdního materiálu. Naše výsledky ukazují, že termoregulační chování je řízeno vnitřními faktory, jmenovitě potřebami kolonie spojenými s kladením vajec a vývojem snůšky. Obě tyto činnosti vyžadují vysokou teplotu.

Mraveniště v odlišných nadmořských výškách se nelišila v průměrné sezónní teplotě hnízda ani denních výkyvech teplot. Variabilita hnízdní teploty byla větší mezi jednotlivými hnízdy na stejné lokalitě nežli mezi lokalitami s odlišnou nadmořskou výškou. Přesto data z ručních měření ukazují, že zde existuje podobnost teplotního režimu mezi hnízdy z nejnižší a nejvyšší nadmořské výšky. Při hledání vysvětlení této zvláštnosti je třeba přihlédnout k jiným vlivům prostředí. Všechna hnízda vykazovala podobně dlouhé období výskytu zvýšené vnitřní teploty ($T>20^{o}C$), přibližně 100 dní, i když počátek tohoto období se mohl mezi jednotlivými hnízdy lišit. Tato studie nalezla průkazný vliv nadmořské výšky na noční změnu teplot uvnitř hnízda ve všech ročních obdobích a také na denní průměr a fluktuaci hnízdních teplot v létě a na podzim. Nadmořská výška ovlivňuje také teploty vzduchu ve všech sezónách, překvapivě na lokalitě s nejvyšší nadmořskou výškou byly nalezeny nejmenší výkyvy teploty vzduchu. Průměrná sezónní teplota vzduchu se nelišila mezi jednotlivými lokalitami s různou nadmořskou výškou.

Klíčová slova:
termoregulace, lesní mravenci, rod *Formica*, vliv nadmořské výšky, inkubace snůšky

ABSTRACT

This study examines thermoregulation behavior of wood ant genus *Formica* on elevation gradient. Two years long dataloger records of inner nest and ambient temperature were combined with detailed spring-summer manual measurement of nest temperature in different depths below nest surface. Results show that inner nest temperature was higher than air temperature in all seasons. Temperature of ant nest is highest in the deepest layer, the heat flows from inside out. We can assume that thermal homeostasis of ant nest in spring and summer i.e. in period of ant highest activity is influenced mainly by inner heat sources – microbial activity and ant metabolism. Insulation seems to have direct effect on nest temperature only in early spring; in summer insulation affects nest temperature indirectly, via ant activity. In winter there is pronounced effect of nest volume which corresponds high isolative properties of nest material. Our results indicate that thermoregulatory behavior of wood ants is driven by endogenous factors, namely colony needs in sense of queen oviposition brood development. Both of these require high temperature.

Nests at variable altitude did not differ in average seasonal temperature or seasonal temperature fluctuation. Variability of nest temperature was bigger among nests from one locality than between localities with different altitude. Yet data from manual measurement show there is similarity in temperature regime between nests from the lowest and highest locality. Possible explanation for this discrepancy should be searched among other environmental factors. All nests show similar length of period with increased inner nest temperature (T>20°C), approximately 100 days, even thought the start of this period may differ among nests. There was found significant effect of altitude onto night change of inner nest temperature in all seasons and also on daily average temperature and temperature fluctuation in summer and autumn. Altitude affects air temperature fluctuation in all seasons too, surprisingly at highest altitude there occurred lower air temperature fluctuations. Average seasonal air temperature did not differ between localities with different altitude.

Key words:
thermoregulation, wood ants, genus *Formica*, effect of altitude, brood development

CONTENTS

I. INTRODUCTION

1. Insect and temperature

Temperature is an important factor for all ectotermous organisms, including ants. The temperature affects all life aspects – for example the rate of development is accelerated with high temperature (Porter 1988), the movement rate speeds up (Challet & col. 2005), the consumption rate of food and oxygen is rising too (Coenen-Strass & col.1980). This means that higher temperature can be advantageous in the sense of higher colony fitness through reproduction and disadvantageous in the sense of energy needs (Brian 1973) at the same time.

The optimal temperature range is variable for different groups of social insect, for example brood of the *Apis melifera* develops fastest at 35°C (Fahrenholz & col. 1989 in Porter & Tschinkel 1993). In F*ormica polyctena* temperature 29°C is preferred for the pupae development (Coenen-Strass 1985), colonies of *Solenopsis invicta* can grow only between 24 to 36°C (Porter 1988). In contrast, the genus *Myrmica* is adapted to cold climates, *M.rubra* from Britain prefer temperatures between 19-21°C, about 8°C lower than the temperature preferred by other ants (Banschbach & col. 1997).

The temperature preferences differ among castes and life stages (Porter & Tschinkel 1993, Rhoades & Davis 1967). Ant queens in *F.polyctena* (Kipyatkov & Shenderova 1986) and *Solenopsis invicta* (Porter & Tschinkel 1993) prefer slightly higher temperatures than workers especially during egg-laying phase, inactive queens may prefer cooler temperatures. Workers generally prefer lower temperatures, which decrease their metabolic rate and increase their lifespan (Ceusters 1977, Porter & Tschinkel 1993); a decrease of 2°C can lengthen worker lifespan in *S. invicta* by 14% (Calabi & Porter 1989). Preferences of nurse workers are shifted to higher temperatures favoring brood development (Kneitz 1966, Brian 1973; Roces & Núñez 1989, Porter & Tschinkel 1993).

Insect societies can achieve much better thermoregulation than solitary insect. This possibility is given by building large and complex nests and by displaying complex behavior (for review see Wilson 1971, Seeley & Heinrich 1981, Johnson & Oldroyd 2007). The nest protects the whole colony and serves as a shelter for adults, and, which is more important, as an incubator for the brood. Temperature in the nest can be achieved stable, which gives an ideal development conditions for brood. This way the reproductive fitness of the colony is raised. Improved homeostasis for the colony could even be one of the reasons why insect sociality evolved (Wilson 1971).

2. Ant thermoregulatory strategies

In moderate climates most ants build nests in the soil where the temperature is quite stable (Hölldobler and Wilson 1990, Sanada-Morimura & col. 2005) or on the soil surface under a layer of leaf litter where the temperature can be buffered by the insulating properties of the nest material. Many species in the Northern Hemisphere also nest under rocks or stones which the ants use as heat collectors (Roces and Nunez 1989, Hölldobler and Wilson 1990, Banschbach & col. 1997, Chen & col. 2002). In the tropics only a few species nest in soil and majority of species inhabit small pieces of rotting wood (Wilson 1971). More precise microclimate regulation is achieved in the mound-building species of *Atta, Acromyrmex, Myrmicaria, Solenopsis, Iridomyrmex, Formica*, and *Lasius* (Brian 1973, Bollazzi and Roces 2002, Cassil & col. 2002, Cole 1994, Coenen-Stass 1985, Frouz 2000, Zahn 1958).

Thanks to huge ant species diversity, there is also a huge diversity of thermoregulatory strategies (for review see Seeley & Heinrich, 1981, Johnson & Oldroyd 2007). Here I would like to mention only information concerning mound building ants, with main emphasis on wood ants genus *Formica*. In general two different thermoregulatory strategies could be distinguishes among mound building ants (Kadochová, Frouz in prep.).

First the nest may increase the available thermal gradient in which the optimal temperature for brood development is selected. Ant workers move the brood (and also the symbiotic fungus) according to the thermal gradients to increase the rate of development. A precise perception of temperature is needed to make the correct choices (Roces & Núñez 1989, Penick & Tschinkel 2008, Bollazzi & Roces 2002). The nest may serve as a solar collector, being heated on one side, whereas the shaded side provides cool shelter for workers, thus prolonging their life-span (Porter & Tschinkel 1993). These nests have usually low thermal capacity and high thermal conductivity, e.g. they can warm up quickly. But they have poor isolation properties. This thermoregulatory strategy occurs for example in *Acromyrmex heyeri, Myrmica rubra, Pogonomyrmex occidentalis, Solenopsis invicta* and genus *Lasius* (Brian 1973, Bollazzi & Roces 2002, Cassil & col 2002, Cole 1994).

Second strategy is to keep stable higher temperature inside the nest. For maintaining stable conditions, bigger nests with good isolative properties are the most suitable (Frouz 1996). These nests warm up slowly but they are able to store gained heat effectively. Ants can regulate thermal loss by moving imides nest aggregation and alternate nest ventilation. Metabolic heat produced by workers (Kneitz 1966, Rosengren & col. 1987) or associated microorganisms (Coenen-Strass & col. 1980) is an important additional source of heat. This strategy can be found for example in honeybees (Lindauer 1954) and other social insect with ability of active thermoregulation, in ant genus *Formica – Formica aquilonia, F. rufa, F. polyctena* (Coenen-Stass 1985, Frouz 2000, Rosengren & col. 1987, Zahn 1958) and *Atta* (Kleineidam & col. 2001), also in fungus-growing termites (Lüscher 1961). The level of nest thermoregulation depends on many other factors, e.g. nest size, population size, moisture and thermal conductivity of the nest material (see below). There are supposed to be intermediate strategies of the thermoregulation.

If the nest is not primary designated for rearing brood the thermoregulation needn't be achieved at all. The secondary nest found in Thatch ants *Formica obscuripes forel* contains only foragers, there are lower temperatures than in the primary nest but still the temperatures are more constant than temperature in the plant canopy. The secondary nest serves as a cool refugee for Homoptera tenders during high midday temperatures and as a primary storage place for honeydew before future transport to the main nest (McIver & Steen 1994). Another similar example is „barrack-nest" of Oecophylla smaragdina, which host only major workers and serve as a base for territory guarding and defense (Hölldobler 1983).

3. Thermoregulation in ants of *Formica rufa* group

3.1 General thermoregulation pattern

Temperature of ant nest is usually higher and more stable than ambient temperature through out the whole year, but in spring and summer the ants maintain markedly higher inner nest temperature than in other seasons (Wilson 1991, Rosengreen & col. 1987, Frouz 2000). High temperatures in spring are required for sexual brood development; nests producing sexual offspring always have higher temperatures than those producing only workers (Luther 1985 in Rosengren & col. 1987). This difference persists even after the sexual offspring have left the nest. During the whole ant activity/ brood rearing period, approximately 100 days (Frouz & Finner 2007), the ants maintain in their nest an area where the temperature is stable and does not drop below 25°C, this place is called heat core (Frouz 2000). Heat core position can be moved according to nest shape and size. There is usually significantly bigger concentration of workers in the heat core than in the nest periphery (Coenen-Strass & col. 1980, Frouz 2000). In winter the nest temperature changes with ambient temperature but temperatures in hibernation chambers remain stable at 1-2°C (Rosengren & col. 1987).

Temperature daily fluctuations in the nest usually positively correlate with ambient temperature (Kneitz 1966, Frouz 2000), but exceptions occur. Rosengreen & col. (1987) reported that a short spell of freeze can increase the inner nest temperature. Daily fluctuations can also be correlated with temperature-dependent changes in ant density and ant aggregations in the central nest. According to Frouz (2000) the highest nest temperatures usually occur in the afternoon or in the evening which corresponds with forager return. These changes in nest temperature could result from the heat brought into the nest by returning workers (heat coming from absorbed solar energy) as well as the heat generated by worker metabolic heat production within the nest. In some nests temperature drops slightly in the morning when ants leave the nest (Horstman 1987, Rosengren & col. 1987, Frouz 2000).

The seasonal fluctuations in thermoregulation behaviour of *Formica polyctena* along south-north gradient were studied by Frouz & Finer (2007). Both in Finland and the Czech Republic the ant colonies maintained a high nest temperature (over 20^{o}C) for a relatively short time period, 65-129 days. This might be explained by the physiology of the queen. Queens of *F. polyctena* undergo regular shifts between reproduction and diapauses, and these shifts seem to be driven by endogenous factors. In the laboratory, the queen enters diapauses after 100 days of reproduction even at a constant temperature and photoperiod (Kipyatkov & Schederova 1990, 1985).

3.2 Outer sources of heat

As mentioned above, the main outer source of heat is solar radiation. The first author drawing attention to the influence of direct solar radiation was Forel in the early 1920s (Seeley & Heinrich 1981). Solar energy help to keep nest material dry (Frouz 2000), thus increases the isolative properties of the nest; it can heat the nest once it is built and it can also increase the metabolism of ants (Kneitz 1966). Compared to underground nests, mounds absorb heat more quickly both in the direct sun and in shade (Penick & Tschinkel 2008). Ants in the Northern Hemisphere usually remove shading grass from the south side so the temperature increases quickly on that side. This creates a temperature gradient that many species use for brood displacement (Penick & Tschinkel 2008). Some species decorate the mound surface with small pebbles or dead vegetation, which can work as heat collectors or as radiation reflectors (Vogt & col. 2008). Mounds of some *Formica* and *Lasius* species could even serve as a rude compass, they are asymmetric, with the main axis oriented in a south–north direction (Hölldobler & Wilson 1990, Frouz 2000, Vogt 2004), the slope of south side negatively correlated with maximal sun angle (Vogt & col. 2008).

Thermal properties of nest material, mainly thermal conductivity and heat storage capacity, may influence solar energy income and heat distribution through the nest. Among others the nest material homogeneity and moisture have the largest effect. In many species there exist aimed differences in the material homogenity and the nest structure. In *Formica rufa* group the organic material is not the same composition in the whole nest volume (Dlusskij 1967, Coenen-Strass & col. 1980). The mound structure is not rigid, the ants loose and renovate the nest structure whereas the material is continuously moved from inside out (Kloft 1959 in Coenen-Strass & col. 1980), sometimes we can observe more compact, dryer layer at the nest surface. An interesting part of *Formica* nest are pieces of resin, incorporated into the nest material. Research of Castella and col. (2008) showed that resin has antimicrobial effect. Resin inhibits the growth of potentially pathogenic bacteria and fungi in the nest. The ants preferently collect pieces of resin to pebbles or twigs, this behavior can be understand as prophylactic (Castella & col. 2008).

Thatch ants *Formica obscuripes forel* (McIver & col. 1997) and *Acromyrmex heyeri* (Bollazi & Rocez 2010a) use plant fragments as building material, and arrange them in a thick compact surface layer called „thatch”. The thatch prevented diurnal nest from overheating by the incoming solar radiation, and avoided losses of the accumulated daily heat into the cold air during the night (Bolaci & Rocez 2010a). This organic material is also expected to have lower thermal diffusivity than the surrounding soil (Cengel 2003), and may therefore prevent heat losses into the soil and so contribute to insulate the fungus garden

Nest moisture can have two different and opposite thermoregulatory effects: moisture can support microbial heat production (i.e. increase the temperature) and decrease the isolating properties of nest (i.e. getting cold quirkier) (Frouz 1996). A study of the relationship between daily temperature regime and moisture in *F polyctena* nests revealed two different situations and possible ways of thermoregulation (Frouz 2000). In the dry nests which are usually located in sunny open places solar radiation plays an important role. Thermal losses of dry nests are estimated to be 0.15–4.3 W per nest (Frouz 1996). These nests have low thermal capacity, but they are usually food insolents. Temperatures of these dry nests are highest in the evening and they drop during the night. Wet nests are usually shaded and thus solar heating is limited. In wet nests the temperatures are low in the evening and increase during the night. These nests have a high thermal capacity and so they require a lot of energy to warm up. High temperatures on nest surfaces at night indicate substantial heat loss, calculated at about 24-30 W per nest (Frouz 1996). On the other hand, bigger nests have more favorable surface-volume ration, which could particularly reduce the heat loss compared to small nests.

Factor contributing to thermoregulation is also solar radiation intake by ant bodies. The ants are dark so they quickly heat when being exposed to the sun during the outside-nest activities. This mechanism was first suggested by Zahn (1958). In spring we can observe ants creating clusters on the mound surface basking in the sun. Their bodies contain great amount of water which has high thermal capacity making the ant bodies an ideal medium for heat transfer. After getting hot enough the ants move inside the nest where the accumulated heat is released. This principle works during all the year but in spring it is most obvious (Rosengren & col. 1987, Frouz 2000).

3.3. Inner sources of heat

As inner sources of heat we can consider heat increasing from microbial activity and ant's metabolism. The thermoregulation via microbial heating was first proposed in 1915 by Wasmann (Wasmann 1945 in Coenen-Strass & col. 1980). In 1980, Coenen-Strass and col. confirmed the existence of microbial heating in ant mounds by showing that in the absence of ants nonsterilized nest material (i.e., with micro organisms) generated heat but sterilized nest material (i.e., without micro organisms) generated almost no heat. The microbial community in the ant nest is different from that in the surrounding soil in part because of differences in pH and food availability and quality (Frouz & col. 2005).

There are detectable seasonal changes in microbial activity; nest material respiration ie. the microbial activity is highest in summer. Heat production per unit of mass is greater for ants than for nest material but concerning the volume ratio of ant bodies to nest volume the total amount of heat produced in a mound is much greater for nest material (Coenen-Strass & col. 1980). Ants can affect the microbial activity via nest material aeration, fresh plant material supply and their own metabolic heat production. Since microbial activity of wet nest material depends strongly on temperature (Coenen-Strass & col. 1980), an increase in temperature in some small parts of the nest (due to ant metabolism or sun radiation) result in an increase in microbial activity and consequently in a subsequent increase in temperature. Microbial activity also significantly rises with moisture (Frouz 2000).

Wood ants of genus *Formica* are able to use metabolic heat to keep proper temperatures inside their nest (Steiner 1924, Kneitz 1964, 1966, Rosengren & col 1987). The temperature inside a *Formica* nest begins to increase very early in the spring, even when the nest surface is covered by ice and snow. At this time the nest could contain larvae, pupae, and even some winged individuals, indicating that the inner heating may have started much earlier (Kneitz 1966). In large nests of *Formica rufa* that contain over 1 million workers, nest heating could start as an autocatalytic process (Rosengren & col. 1987) that relies on utilizing of lipid reserves in young workers (Martin 1980).

Some authors deny the importance of ant metabolic heating capacity for thermoregulation in nests of wood ants. Review by Seeley & Heinrich (1981) places the main emphasis on the red wood ant mound as a solar collector and threats the part played by metabolic heat as a rather uncertain. Brandt (1980) who studied the thermal diffusivity of nest material in wood ants came to conclusion that “there is no need to introduce ants with physiological heat production”. Coenen-Strass & col. (1980) measured thermal productivity of nest material, workers and pupae of wood ants *F.polyctena* and assumed that the heat produced by ant metabolism does not play an important role. His results indicate the microbial activity of nest material to be the main source of heat production.

However a study done by Rosengren & col. (1987) showed results that cannot be explained by above mentioned theories only and support the existence of ant metabolic heating. First he found a negative correlation between inner nest temperature and ambient temperature in spring, which doesn’t fit the insulation hypothesis, but could result from ant workers producing metabolic heat clustered together in the centre of the nest when ambient temperature drops below threshold limiting outdoor activities. Rosengren & col. (1987) recorded a significantly increased temperature, nearly 25^{o}C, in wood ant nest during period when ambient temperatures were only +0.5 ^{o}C, and the nest was still covered by ice crust. Thus neither insulation nor increased microbial activity through intensive ant building behavior (ants were kept inside the nest by the ice layer) could count as explanation. Similar results were shown by Frouz & Finner (2007). It is also difficult to explain the maintenance of fixed target temperature favoring brood development and buffered against thermal fluctuations without ant metabolic heating (Rosengren & col. 1987).

Recent studies usually assume that thermoregulation in wood ant nest is achieved by combination of all mechanisms (Frouz 2000, Jones & Oldroy 2007).

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II. GOALS AND HYPOTHESIS OF STUDY

The maintenance of a stable temperature in wood ant nests during the period of ant activity is widely known and has been the subject of many studies (Steiner 1924; Raignier 1948; Dlusskij 1967; Galle 1973; Hostmann 1983, Horstmann & Schmid 1986; Frouz 1996, 2000). Much is known, but still the results are not in perfect agreement. We can say the more we know, the bigger discrepancies there are, the more questions arise.

This study investigates the thermoregulation behavior in wood ants *Formica polyctena* on the elevation gradient. The aim of my diploma thesis is to add new knowledge to today thermoregulation paradigm, and if possible, to find support for one or more of earlier hypotheses. The study was designed according to former study of Frouz & Finer (2007) investigating thermoregulatory behavior along s south-north gradient. The methodic was planned to allow comparing results of this two studies. New working procedures were added to obtain data about other factors that are expected to influence nest temperature. Study is based on long term automatic monitoring of ant nest temperatures. During detailed manual sampling I additionally measured the insulation level and ant activity.

Main research question was:

- **Does the altitude affect the thermoregulation behavior of wood ants?**

We were interested in these factors: the possible difference of average, maximal and minimal inner nest temperature among nests along elevation gradient, the beginning and length of period with increased inner nest temperature, the differences in thermoregulatory behavior among individual nests, thermoregulation behavior stability during the whole year, differences in nest temperature dependence on ambient temperature in spring, summer and autumn, other factors influencing thermoregulatory behavior.

Our zero hypotheses about thermoregulation in genus *Formica* were**:**

- **Thermoregulation behavior is not dependent on the elevation gradient.**
- **Temperature fluctuation is not correlated with elevation gradient.**
- **Dependence of inner nest temperature on the ambient temperature does not differ in spring and in autumn.**

III. MATERIAL AND METHODS

1. Study site

The study was performed in the Czech Republic, in South Bohemia nearby a village Srní (49°04'51", 13°28'44") which lies in western part of National Park Šumava (figure 1). National Park Šumava was established in 1991, it covers area of 680 square kilometers, it is part of UNESCO biosphere reservations in program Man an Biosphere, Natura 2000 and The Ramsar convention on Wetlends. Šumava is one of the oldest mountain range in central Europe with average heights of 800–1400 meters, the highest peak is Plechý Mountain 1.378m. The area is covered mostly by coniferous forest dominated by Norway spruce (*Picea abies* L.Kaster). There are well preserved ecosystems representing the natural state of mountain forest habitat in the temperate zone, thus the study should reveal a natural patterns of ant ecology.

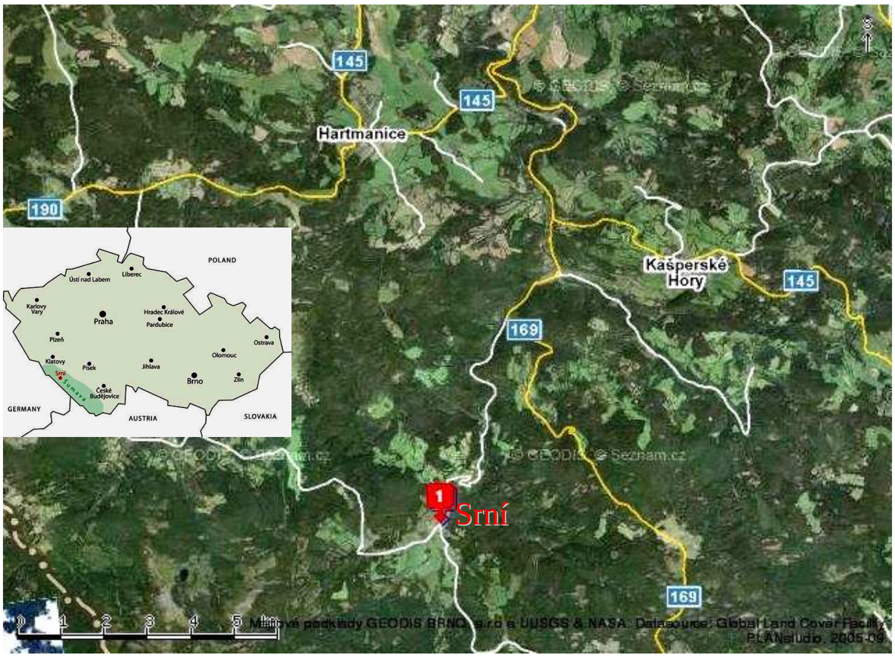


Figure 1. Photomap of western part of National park Šumava with marked site of study nearby village Srní; on left side map of Czech Republic with position of Šumava Mountains. Source of map Google.com

The studied nests occurred in coniferous forest habitat dominated by spruce, the forest is older than 60 years with sunny patches of deforested habitat, changing continuously to the meadows. The study was performed along a hillside of one mountain, going from the valley to the top, the area was divided into three localities marked A, B, C (figure 2) with different altitude. We wanted to limit the differences between studied localities in ambient conditions, including habitat type, weather conditions, average temperature and precipitation etc. and also in between genetic composition of wood ant community, to those caused by altitude only. Locality A was the lowest one, with average altitude 816 m, it was situated in valley of Hrádecký potok spring boarding with first zone of Vydra river (intervention free area). Locality B has an intermediate altitude, it was in spare forest situated at the end of open plain with average altitude 858.7 m. Locatity C has the highest altitude, in average 1068.8 m, it was situated on the hillside of Zelená Hora Mountain (1239 m).

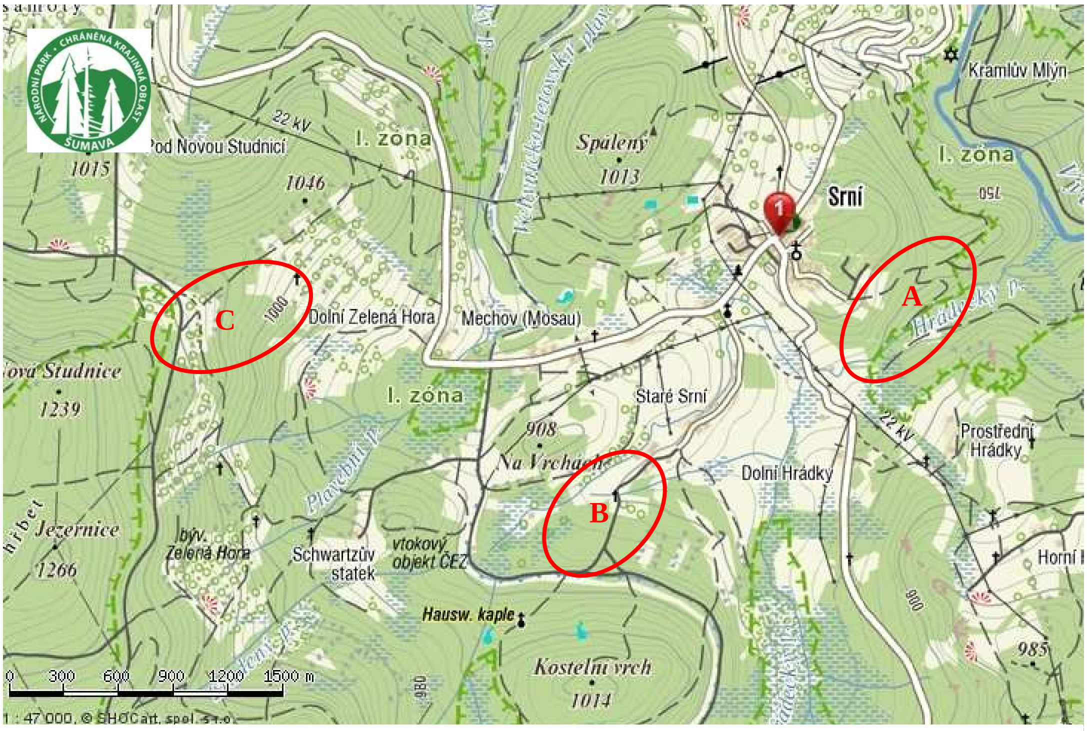


Figure 2. Detailed map of study site nearby village Srní, elevation level and vegetation cover pictured: green color for forest cover, white for open spaces. Localities A, B, C are marked with red elipsoid.

The sites were inhabited by polydomous colony of wood ants of *Formica rufa* group. In total twelve ant mounds were studied, four mounds of different size representing each locality A, B, C. The position of every nest was located with GPS system (Trimble GeoTX) allowing proper mapping and altitude location. Physical characteristics of the mound, i.e. mound size, nest material moisture, level of nest shading, were measured prior to the continuous data collection. All nest measured in our study showed high survival rate except one, nest B1, which was particularly abandoned in spring 2011. Only a small part on mound near apex was inhabited by ants, the rest of mound was wet and grown through by fungi and mildew. In all other nest a massive ant building activity and swarming was observed every spring. The study duration was 23 months in total.

2. Data collection

2.1. Continuous temperature recording with datalogers

Inner nest temperature was recorded using digital dataloggers (T174, Testo, Germany). This compact apparatus (1x3x5 cm) is accurate to 0.1°C and stores up to 2000 data entries. The dataloggers were wrapped in plastic foil and tape to minimalize the apparatus corrosion. One datalogger per nest was used. The dataloggers were inserted into the center of ant mound at 1/3 under the mound apex, where they stayed for the whole recording time. This position was used because it approximated the heat core of the nest, where ants maintain a constant temperature (Frouz 2000). Little movement inside the nest interior is possible due to the ant activity. The datalogger was attained with a plastic rope to the peg outside the nest to allow future extraction with minimal nest damage. At each of three localities A, B, C one dataloger was also placed outside the nests to measure the ambient temperature; it was attached to a tree twigs 30 cm above ground, the place being protected from direct sun radiation.

The datalogers were placed into the selected ant mounds on 24th may 2009 and they collected temperature data every 3 hours for the whole year, this means 8 data entries a day. After this time the datalogers were replaced with second set of datalogers. Data from Kilpeläinen (2008) show that ants are able to repair the nest after mechanic disturbation in a single week, thus we can assume that thermal homeostasis of nest wasn't harmed with the datalogers replacement. In total two years data were collected, the very last data were recorded on 22nd April 2011, than the dataloggers were extracted from all nests.

From the first season of measurement we are missing three datalogers (A2, B1, C1). The holding rope of these apparatus was chewed by ants and the datalogers were moved somewhere deep inside the nest, may be underground. We weren't able to detect these lost dataloggers with metal detector and we did not want to destroy the whole colony of ants (belonging to animals protected by law) during digging through the underground nest. Data from datalogger B4 were damaged thanks to massive apparatus corrosion. It means that in first season we lost four of twelve nest dataloggers. We also lost one datalogger recording ambient temperature at location A. Fortunately in second years the ants were not so aggressive and all datalogers placed inside nests were successfully extracted, also all outside dataloggers remained untouched.

Prior to data collection physical characteristics of the mound were measured: nest high and diameter, which were later used for calculating nest volume. Volume of nest was approximated as a volume of a cone, the same method was used in study of Frouz 2000. Nest material moisture was measured gravimetrically, the difference of weight between wet and dry material, desiccated for 6 hours in electric oven at 40°C, was counted. The samples for moisture measuring were taken from the nest interior at the 1/3 depth from the apex, a place were the datalogers were later inserted. The data about average daily precipitation (in mm) were obtained from Czech hydro meteorological institute. For characteristics of individual nest see table 1 in appendix.

2.2. Spot sampling by hand

In summer 2009 a pioneer measurement was carried to test the experimental set-up. Continuous spot sampling by hand was carried in the season 2010, in the period of ant activity from April to September. The nests were sampled once each month, in the last week of month, i.e. there were six sampling periods in total. I spent one day measuring nests at each locality A, B, C, i.e. three following days for a month. The idea was to perform the sampling always in three directly following days to insure similar weather conditions in all nest groups. The sampling was carried out in all weather conditions.

The temperature data collection was performed five times a day: one hour before the sun rise, one hour after the sun rise, in the middle of photophase i.e. around midday (the time of highest solar income), one hour before the sun set, one hour after the sun set. The time was not the same during the whole sampling period, because the sun set/rise time is changing during the year. We were not interested in the concrete time, more important for our study was the income of solar energy connected with sun position in the sky and length of photophase. The longest days were in June (exactly on 21st June 2010), earliest measurement was done at about 4 AM and the latest at about 11 PM.

The spot sampling was conducted with mercury thermometers (accuracy to 0.1°C) inserted into the ant mound apex at four different depths: on the surface of the mound, 5, 10, 15 cm below the surface. The very same thermometer was always used for measuring the same depth. The temperature on the surface of mound was taken from place 10 – 20 cm above ground level, if possible in a shadow. At few occasions there was no shadow at all on the nest surface, nevertheless the temperature was sampled. The thermometers were placed inside the nest for at least three minutes to stabilize.

Every time during the spot sampling following data were additionally collected: the solar insulation level with Luxmetr (LX-1108, Voltcraft), number of entrances onto the mound apex in the circle with diameter approximately 30 cm, and number of foragers on the trail per minute. Short part of trail was selected and marked with two sticks, between these sticks all workers in both directions were counted in one minute time span. The counting was repeated twice. The number of ants leaving or returning the nest and number of nest entrances opened were used for calculating the ant activity. The instant insulation data were used for counting average nest insulation per season.

2.3. Continuous ten-hour measurement

In July, in the period of highest ant activity, a continuous ten hours measurement was done. This measurement was aimed to study the overnight changes in inner nest temperature and possible heat losses via nest surface. Temperatures from datalogers recording nest core temperature and ambient temperature were added to the analysis. The nests were first sampled at 2 PM and since that every hour until 12 PM, additionally temperature at 4AM and 6AM was measured. The sampling procedure was the very same as during the regular month spot sampling by hand; solar income, number of nest openings and number of foragers walking was recorded (see the previous paragraph). On 27th July nests in the group A (the lowest one) were measured, two days after nests in group B were investigated. But the weather went wrong, it was heavy raining all the time so performing the measurement was disabled. The continuous measurement was repeated on 1st and 2nd August in group B and C respectively. Still most of 10hour sampling was carried out in rainy conditions. All measurements were done by one person, so the interpersonal mistakes should be minimalized.

3. Data processing

3.1. Missing data

Missing data from dataloger recording ambient temperature at locality A (May 2009 – September 2010) were replaced with temperature data from locality B. These localities are close to each other, the average temperature difference is between locality A and B in 2010/2011 was 0.88°C. The cases when the temperature difference was lower than 0.5°C counted 53% of all cases. The differences bigger than 3°C created only 5.9% of all cases, the biggest differences appeared in November and January, which is the part of year when ants are not active. We can thus assume that the replacement of ambient temperature A with ambient temperature B in our study did not cause a great difference. From 1st October 2010 the data from locality A are available.

Missing data from datalogers recording inner temperature in the first year of study (May 2009 - June 2010) inside nests A2, B1, B4, C1 were not replaced. For statistic we use only data from second set of datalogers recording the temperatures from July 2010 to April 2011. Dataloger data from the day of datalogers replacement were not included into the study.

3.2. Statistical software used in analysis

Data were exported from the dataloggers to Excel spreadsheets using Testo Basic software (Testo, Germany). Together we have more than 5700 data records for each of twelve nests. Microsoft Excel was also used for data manipulation, and calculation of means, SD, and fluctuation of daily inner nest temperature (difference of maximum and minimum daily temperature) and numbers of days with mean temperature over 20^{o}C or lower than 0^{o}C. Most of figures were also created in Excel.

Statistic program R 2.11.1 was used for statistical modeling and testing environmental factor effect onto nest temperature. General linear models were used to evaluate the most important factor explaining the daily average nest temperatures, daily temperature fluctuations and night temperature change. Models were always counted with backward selection. To evaluate factor importance, the sum of squares explained by a given parameter was expressed as a percentage of the total sum of squares. The difference in nest temperature between 24:00 to 3:00 hours was used as a measure of night time nest temperature change (Frouz, 2000). This period in the middle of night was between sunset and sunrise in all cases, so insulation effect onto nest thermoregulation was negotiated.

Correlation between number of days with inner nest temperature $T>20^{o}$C, $T<0^{o}$C during the whole year, first day of spring heating with $T>20^{o}$C and environmental factors was first counted by multiple regression statistical program Instat3 and than a linear model in R program was tested. Results and correlation values given by both program were identical. The easiest possible model was fitted, with additive effect of factors only. Statistical differences among nests temperatures between measured localities and between individual seasons were counted with non-paired, two side t-test.

ANOVA was used to compare datalogger data about nest temperature between various seasons, locations and nests; also for comparing data from spot sampling by hand according to nest, day time and date of sampling. To evaluate long term trends in temperature in winter, spring, autumn and summer periods, slopes of temperature changes for individual nests and air temperature were calculated by linear regression. Slope differences were compared using the confidence interval of these slopes. ANOVA and correlations between individual variables were computed using SPSS 10.0. Ant activity and factor importance were counted and visualized by Canoco for Windows 4.5.

IV. RESULTS

1. Whole year temperature regime

1.1. Seasonal averages of nest and air temperature

Average inner nest average temperatures were significantly higher than ambient temperatures in all seasons. In most cases there was significant difference of average seasonal inner nest temperature both among individual nests and in one nest between two measured years (Tukey HSD test, $p<0.001$). Nevertheless the temperature regime in both years was similar (figure 3). The inner nest temperatures reached maxima during late spring or during summer, in winter the inner nest temperatures were oscillating close to zero. Spring and autumn average nest temperatures were similar; the value was in between summer and winter temperature (table 1). Seasonal average ambient temperatures in localities A, B, C show no significant differences among all seasons and years (Tukey HSD test, $p<0.001$).

In spring average inner nest temperature pooled for all nests was 10.80°C in spring 2010 and 5.44°C in March-April 2011, whereas the average ambient spring temperature was 4.20°C and 3.35°C in 2010 and 2011 respectively. Average inner nest temperatures in March-April 2011 were similar to average ambient temperature. The spring temperature pattern was not uniform throughout all spring, early spring nest temperatures were low, fluctuating around 5°C, than a steep temperature increase was observed. The timing of spring temperatures increase differed among individual nests (for more details see chapter 1.4.). The maximal and minimal inner nest temperature recorded in spring was 30.3°C and -5.7°C respectively. Maximal and minimal ambient temperature was 28.0°C and -21.1°C respectively. Freezing temperatures occurred more often in March.

From May onwards a stable inner nest temperature plateau was reached with temperatures significantly higher than ambient temperatures (t-test, $p=0.036$). Summer inner nest temperatures were nearly two times higher than ambient temperatures in all nests in both years with exception of nest B1 in summer 2010. The average nest temperatures were very high both in summer 2009, in average 24.39°C pooled for all nests, and in summer 2010, in average 23.55°C. Maximal and minimal recorded nest temperature was 31.6°C and 6.8°C respectively. Ambient summer average temperature was 13.44°C in summer 2009 and 13.77°C in 2010. Maximal air temperature reached 31.0°C, minimum was 0.2°C. In 2009 temperatures of nest at Zelená hora mountain (locality C) were statistically most homogenous (Tukey HSD test, $p=0.00$), although two nets form other localities (B3, A4) showed similar characteristics (table 1). No significant differences were found in average summer inner nest temperature (pooled for all summer) among nests in localities A, B, C.

During autumn inner nest temperatures were steadily decreasing, in 2009 there was recorded a big drop of inner nest temperature, approximately 15-20°C, in a single week in the first half of October (8.-15.10.2009). In autumn 2010 the temperature decrease was more gentle, except of nests C3 and C4 which also shoved rapid temperature drop. Autumn average inner nest temperature was 12.32°C in 2009 pooled for all nests and 10.54°C in 2010. Ambient average temperature was significantly lower, 12.32°C and 10.54°C in 2009 and 2010 respectively. Autumn average temperatures differed among individual nests; there were no significant differences among localities with different altitude. Maximal recorded autumn inner nest temperature was 29.0°C, minimal temperature was -2.2°C. Air maximal and minimal temperature was 27.1°C and -15.9°C respectively.

In winter a plateau with stable temperatures was reached, average temperatures were 0.30°C in winter 2009/10 pooled for all nests and 0.54°C in winter 2010/11. Comparing the two measured winter seasons the inner nest temperature of individual nests in 2011 was higher than in 2010 for more than half of nests. In 2009/10 averages for inner nest temperature were similar in all nests and significantly different form ambient temperature (Tukey HSD test, $p<0.001$). In winter 2010/11 there occurred significant difference of nest temperature between locality A and B (t-test, $p= 0.033$). Ambient winter average temperature was -3.55°C and -3.20°C in 2009/10 and 2010/11 respectively. Maximal recorded winter nest temperature was 8.7°C, minimal temperature was -7.2°C. Ambient winter temperatures reached maximum 16.2°C and minimum -24.0°C.

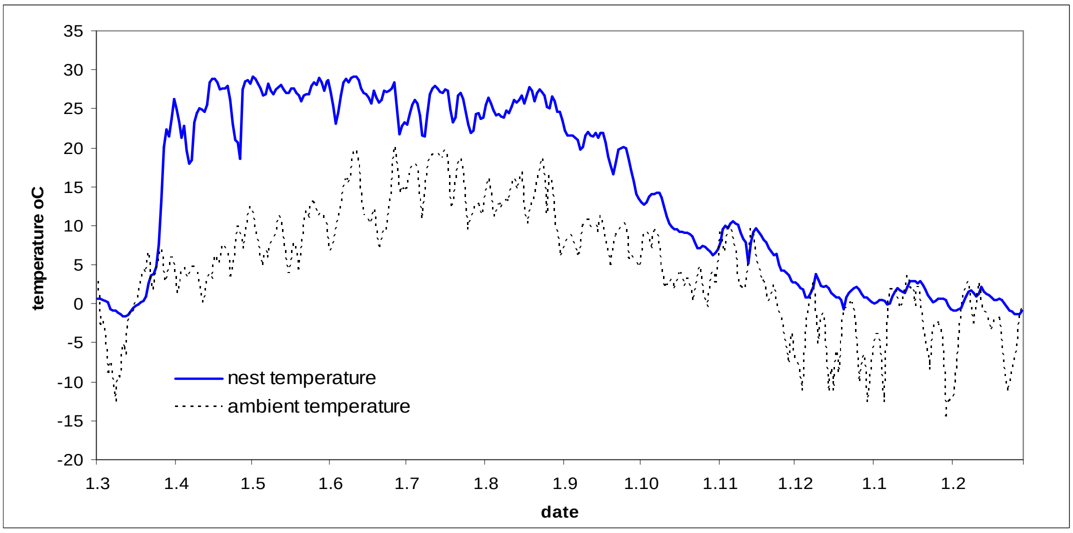


Figure 3. Typical whole year pattern (spring 2010 – winter2010/11) of inner nest temperature changes compared to ambient temperature changes. Data given for nest A1, whole year datalogger records.

nest/air	mean	±SD	group	mean	±SD	group	nest/air	mean	±SD	group	mean	±SD	group
SPRING	2010			2011			SUMMER	2009			2010		
A1	18.93	11.668	j	9.66	9.062	efghi	A1	28.23	0.833	l	25.81	1.829	k
A2	-			5.53	7.486	abcdef	A2	-			26.04	1.988	k
A3	9.52	4.250	eghi	4.22	8.405	abcd	A3	22.02	2.693	defg	20.92	3.497	cde
A4	7.95	6.542	cdefghi	4.56	5.819	abcdf	A4	23.87	2.544	hij	23.76	2.724	ghi
air A	4.39	5.536	abc	3.29	4.177	ab	air A	13.58	2.793	a	13.85	3.488	a
B1	-			3.81	3.181	abcd	B1	-			18.65	2.256	b
B2	7.54	6.668	bcdefgh	3.89	4.261	abcd	B2	20.14	2.824	bc	21.23	3.866	cdef
B3	11.88	9.071	h	7.04	5.824	abcdefgh	B3	25.42	3.066	lnk	24.74	2.294	ijk
B4	-			2.71	3.674	a	B4	-			24.70	1.752	ijk
air B	4.39	5.536	abc	3.47	4.126	abc	air B	13.58	2.793	a	13.74	3.428	a
C1	-			9.03	8.431	defghi	C1	-			20.39	1.982	bcd
C2	10.05	7.851	eghi	5.64	4.824	abcdefg	C2	25.21	3.367	lnk	22.58	2.806	efgh
C3	10.26	10.886	ghi	-			C3	24.64	5.015	lnk	24.10	5.445	hijk
C4	10.28	10.415	hi	3.00	6.087	ab	C4	25.58	2.898	jk	22.76	4.961	fgh
air C	3.82	4.484	ab	3.30	4.472	ab	air C	13.17	3.295	a	13.72	4.323	a
AUTUMN	2009			2010			WINTER	2009/10			2010/11		
A1	14.70	7.514	h	12.37	5.884	efgh	A1	0.52	2.373	RGB	0.98	1.134	gh
A2	-			12.93	5.273	fgh	A2	-			2.60	0.811	i
A3	10.08	5.188	cdef	8.31	4.122	bc	A3	-0.05	1.700	defgh	0.48	1.596	fgh
A4	9.29	4.538	bcd	8.71	3.871	bc	A4	0.39	1.782	efgh	1.32	1.137	hi
air A	6.85	4.728	ab	4.92	4.194	a	air A	-3.51	4.263	ab	-4.14	4.703	a
B1	-			9.29	3.850	bcd	B1	-			-0.27	1.264	defg
B2	10.22	5.339	cdefg	8.64	3.882	bc	B2	-0.92	2.115	cde	-0.56	1.160	def
B3	13.92	6.372	h	13.11	6.169	gh	B3	0.76	2.864	RGB	-0.02	1.534	defgh
B4	-			10.82	4.817	cdefg	B4	-			-0.27	1.455	defg
air B	6.75	4.670	ab	5.01	4.362	a	air B	-3.51	4.263	ab	-3.12	4.138	ab
C1	-			9.39	4.930	bcde	C1	-			1.07	0.703	gh
C2	14.46	7.725	h	10.62	5.493	cdefg	C2	0.23	2.223	defgh	-0.99	2.166	cd
C3	12.85	8.076	fgh	11.94	5.910	defgh	C3	0.94	2.398	gh	1.02	0.872	fghi
C4	13.01	7.557	fgh	10.31	5.372	cdefg	C4	0.54	2.456	RGB	0.05	1.503	defgh
air C	6.43	4.997	ab	4.89	4.356	a	air C	-3.63	4.007	ab	-2.32	3.289	bc

Table1. Seasonal nest and air temperature averages ±SD, temperatur eis given in ^{o}C. Significantly homogenous groups are marked with same letter (ANOVA, Tukey, HSD test $p<0.05$ for all cases). Comparison is based on dalaloger data daily averages

1.2. Slopes of seasonal temperature change

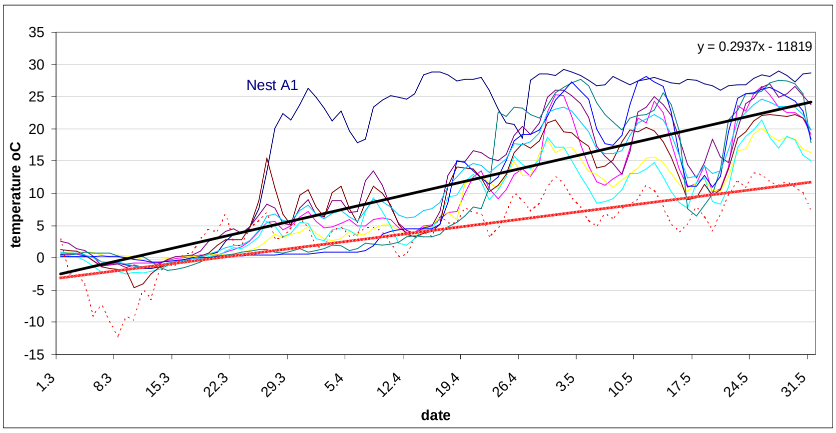
Whole year pattern of nest temperature changes showed some similarity to pattern of ambient temperature changes, but still there were important differences (figure 4). In spring the air temperatures increased, so did the nest temperature. But the slope of nest temperature changes was much steeper (0.309 pooled for all nests and both years) than that of air temperatures (0.176). Comparison of slope confidence intervals ($p<0.05$) showed that all nests in 2010 had significantly steeper slopes than slopes of ambient temperatures in given location , in spring 2011 this was true only for half of the nests, other nests showed slopes identical with ambient temperature change. Again we have to mention that for spring 2011 data from May are missing.

In summer both slopes of nest temperature (0.013 pooled for all nests and both years) and air temperatures (0.021) was close to zero, indicating no important temperature changes. In summer 2009 the general trend was slightly increasing most of nests showed slopes identical with ambient temperature change, except of nest A1, A3 which had lower slope and nest C3 with slightly higher slope ($p<0.05$). On contrary in summer 2010 the general trend was shifted towards negative values, indicating slight decrease of both air and nest temperature during summer. In 2010 all nests except A2 (lower slope) showed identical slopes with slope of air temperature changes. But there was a significant difference in inner nest temperatures, which were two times higher that air temperatures (Tukey HSD test, $p<0.001$).

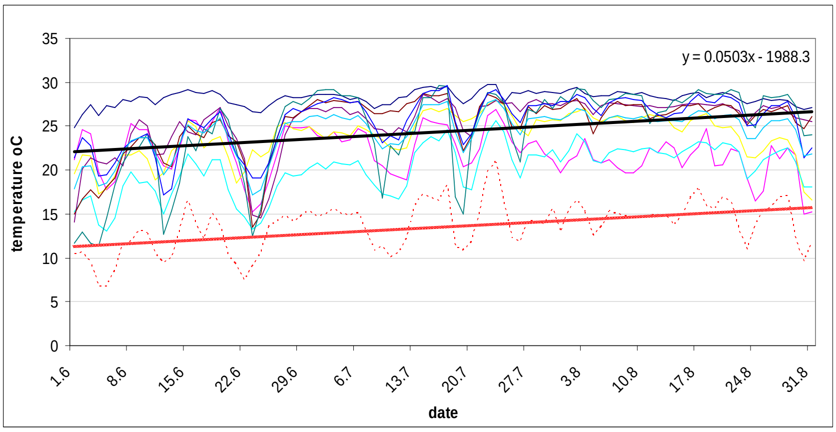
In autumn we could observe decreasing slope of both nest and air temperatures, the slope of nest temperature changes (-0.183 pooled for all nests and both years) was much steeper than slope of air temperature changes (0.110 pooled for all locations an both years). In autumn 2009 all nests except of A4 showed lower slopes than air temperatures, in 2010 half of nest showed lower slopes and half had slopes identical with ambient temperature change.

In winter both slope of nest and air temperature change was close to zero, yet nest temperatures were only slightly decreasing (slope -0.038 pooled for all nests and both seasons) while slope of air temperatures slightly increasing (0.017 pooled for all locations an both years). Nevertheless the difference between air and nest temperature slopes was not significant, the confidence intervals for all nests temperature slope and air temperature slopes did overlap. In other words all nests showed identical slopes of temperature changes compared to slope of ambient temperatures in both winter 2009/10 and 2010/11 (tab.2 in appendix). There were significant differences of average nest and air temperature ($p<0.001$).

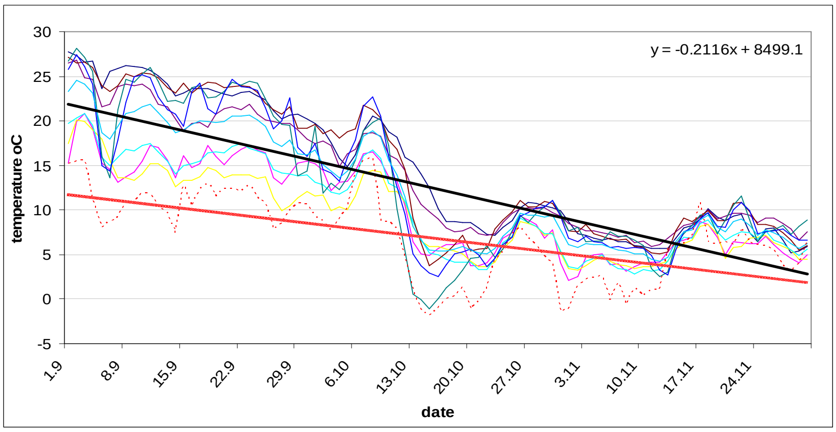
Figure 4. Slopes of seasonal nest temperature changes compared to air temperature changes, the linear trend connector line of nest temperature is given in black color for all nest pooled together with line equation, linear trend connector line of air temperature is given in red color.



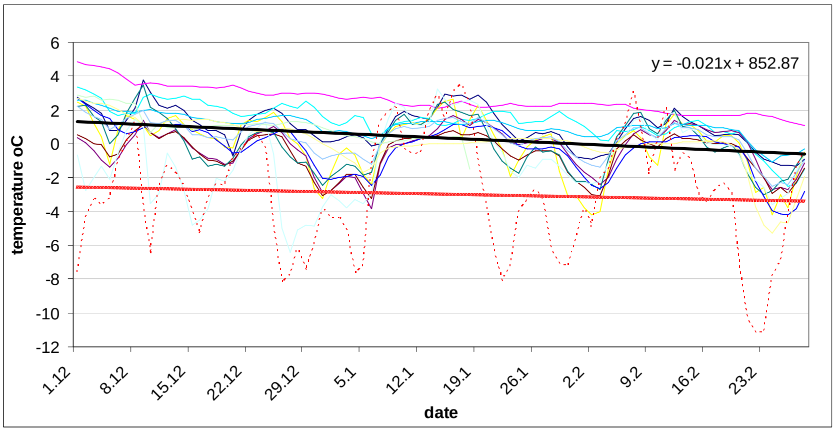
Spring
1.3.-31.5.2010



Summer:
1.6.-31.8.2009



Autumn:
1.9.- 30. 11.2009



Winter:
1.12.2009 – 28.2.2010

1.3. Factors influencing average seasonal temperature

Because of great differences in nest temperature patterns inner nest temperature were counted separately for individual seasons using linear models. Plots concerning individual seasonal models can be found in appendix (figure 2-3). In all seasons nest identification and date were among the most important factors. The nest shares all characteristics such as nest volume, moisture, altitude, shading and insulation level. Each day has unique level of factors such as air or nest temperature and fluctuation and precipitation. But there is still enough variability left to be explained by other factors.

In summer three most important factors explaining average inner nest temperature are mean air temperature, nest identification and date, all of them with significance $p<0.001$. Other important factors are year, altitude, nest volume and moisture and precipitation. Only nest shading is not significant. This model explains 67.42% of all variability found in summer average temperature.

In winter three most important factors explaining average inner nest temperature are again nest identification and mean air temperature, this time together with year. Other significant factors are nest moisture, date and air temperature fluctuation, all of them with $p<0.001$. Nor nest moisture neither altitude show significant effect. This model explains 48.38% of all variability in winter inner nest average temperature.

Response variable: **summer average nest temperature**			
Factor	Sum Sq	F value	p
mean air temperature	8362.2	1654.3384	***
nest	7702.2	138.5246	***
date	629.8	124.5942	***
year	539.7	106.7777	***
volume	328.5	64.9963	***
air temp. fluctuation	222.9	44.0984	***
altitude	37.1	7.3317	**
rain	36.8	7.2785	**
moisture	20.2	3.9929	*
residuals	8638.5		

Table 2 – factors explaining average inner nest temperature in summer. Factors ordered “ according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

Response variable: **winter average nest temperature**			
Factor	Sum Sq	F value	p
nest	1468.2	762.166	***
year	844.6	39.858	***
mean air temperature	454.6	236.012	***
moisture	97.8	50.757	***
date	80.9	42.002	***
air temp. fluctuation	36.8	19.081	***
residuals	3182.3		

Table 3 – factors explaining average inner nest temperature in winter. Factors ordered according to explained sum of squares. Significance codes: >0.0001 ‘***’ 0.001 ‘**’ 0.01 ‘*’

In spring three most important factors explaining average inner nest temperature are year, date and nest identification, all of them with significance p<0.001. Other important factors are mean air temperature, nest moisture and precipitation. Neither nest volume, altitude nor nest shading is significant. This model explains 81.45% of all variability found in spring average temperature.

In autumn three most important factors explaining average inner nest temperature are year, mean air temperature and nest identification, all of them with significance p<0.001. Other important factors are date, altitude, nest moisture and air temperature fluctuation. Neither nest volume nor nest shading is significant. This model explains 73.88% of all variability found in autumn average temperature.

Mean air temperature correlates to nest temperature significantly in all seasons. Nest moisture was significant in all cases, whereas nest volume only in summer. Rain played an important role in spring and summer. Altitude effect onto inner nest average temperature was significant in half of cases, namely in summer and autumn.

Response variable: **spring average nest temperature**			
Factor	Sum Sq	F value	p
year	28805.7	1887.555	***
date	23077.1	1512.180	***
nest	9419.6	56.113	***
mean air temperature	3582.2	234.730	***
moisture	501.8	32.879	***
rain	212.2	13.904	***
residuals	14940.4		

Table 4 – factors explaining average inner nest temperature in spring. Factors ordered according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

Response variable: **autumn average nest temperature**			
Factor	Sum Sq	F value	p
year	38681	6492.5727	***
mean air temperature	6889	1156.2840	***
nest	5252	88.1572	***
date	4443	745.7888	***
altitude	439	73.6137	***
moisture	92	15.4401	***
air temp. fluctuation	36	5.9659	*
residuals	19742		

Table 5 – factors explaining average inner nest temperature in autumn. Factors ordered “ according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

1.4. Spring temperature increase

In early spring the inner nest temperatures were low, fluctuating around 5°C, than a steep temperature increase was observed. In few days nest temperatures rose to 20°C or more, the temperature increase could be more than 10°C a day, the biggest daily temperature increase was recorded on 29.3.2011 in nest C1 (for more details see chapter 2.2.). The steep temperature increase was preceded by period of mild temperature increase in some nests. The nest temperature increase happened from end of March to end of April, the timing of inner nest temperature increase was significantly different both among nests and seasons.

Table 6 shows the first spring day with temperature exceeding 20°C for both years, the value of inner nest temperature is given. We can see exceptional values of nest A1 which was the very first in 2010 spring increase, it happened on 27.3. Other nests were one month late, first day with T>20°C occurred at the end of April, in nest A4 at the end of May. In 2011 the first nest with average temperatures exceeding 20°C was nest C1 on 30.3., most nests showed increased temperature in second half of April. Neither differences in first day with T>20°C nor differences in first day with temperature change >5°C among nests in localities A, B, C were statistically significant. In 2011 spring increase of most nests was shifted a little bit further compared to spring 2010; unfortunately we do not have complete data for spring 2011 (May is missing), so the comparison between two consecutive years is disabled. Thanks to extraction of dataloggers on 22nd April 2011 we did not catch the spring temperature increase of most nests. Namely no nest at locality B showed T>20°C, at localities A and C half of nest showed temperature increase earlier than datalogger extraction (table 6).

year	**2010**	**2011**		**2010**	**2011**		**2010**	**2011**
A1	27.3. 20.15°C	4.4. 21.0°C	**B1**	-	>22.4	**C1**	-	30.3. 23.2°C
A2	-	15.4. 22.49°C	**B2**	25.4. 21.36°C	>22.4	**C2**	29.4. 20.99°C	>22.4
A3	29.4. 22.64°C	>22.4	**B3**	26.4. 20.35°C	>22.4	**C3**	23.4. 22.58°C	>22.4
A4	25.5. 20.05°C	>22.4	**B4**	-	>22.4	**C4**	29.4. 21.9°C	21.4. 20.28°C

Tab 6. First spring day with daily average nest temperature >20°C; if coming later than datalogger extraction on 22.4.2011 the days are marked with “>22.4.”. Missing data from year 2010 marked as “-”. The exact temperature of each nest is given in °C.

When looking for factors explaining the timing of spring temperature increase counted as first spring day with T>20°C we found significant effect of average spring inner nest temperature, average spring air temperature, nest insulation and number of ants walking (factors ordered according to amount of explained variability). Last two values were obtained from summer season sampling by hand. The best model explaining spring heating of nest was: [h.day] = 313.54 - 0.009814*[sun] + 0.01727*[ants] - 5.945*[nest.av] + 101.35*[air.av]. This model explained 95.35% of all variability and was significant with p=0.024. Neither altitude nor nest volume and moisture effect was significant in context of spring heating. When deleting average inner nest temperature from the model all other factors lost their significance and there was no factor with significant effect left.

Heating of nest A1 (figure 5) started at the end of March in 2010, a steep increase was recorded on 26.3.2010 between 11 AM (t = 9.8°C) and 5 PM (t = 18.1°C), which means an increase of 1.38°C per one hour. In consecutive days the temperature continually raised to maximum of 27.1°C, which occurred on 31.3.2010 whereas the ambient temperature was only 3.5°C; from this day onwards the inner nest temperatures stayed close to 25°C. The increase in inner nest temperature was preceded by great ambient fluctuations with morning temperatures close to -4°C and afternoon air temperatures coming up to 20°C. It can be seen that short spell of cold temperatures of even freeze didn't have negative effect onto nest temperature (see figure 3, week 9.-16.4.20010). Although longer period of strong air temperature fluctuation can cause a drop in inner nest temperature. In nest C3 the spring steep temperature increase was shifted to end of April, it occurred on 22.4.2010, which is a month later than in nest A1. Temperature raised from 6.8°C on 22nd midnight to 21°C on 23rd midnight, which means an increase of 0.49°C per hour.

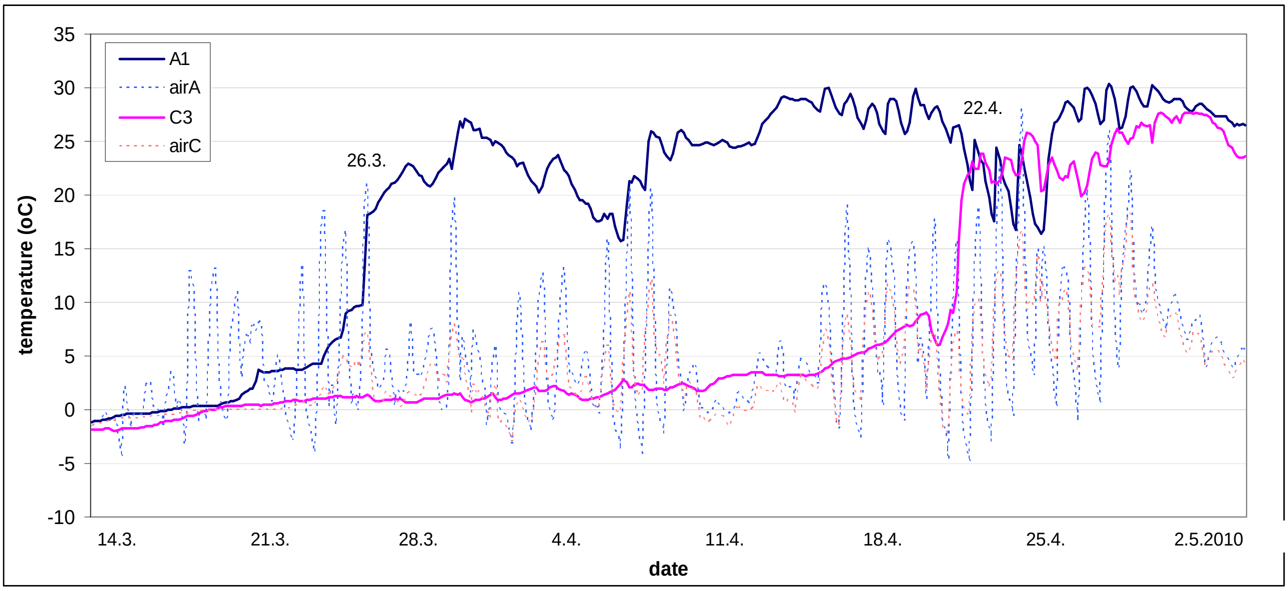


Figure 5. Timing of spring increase of inner nest temperature in nest A1(altitude 856.4m above see level) compared to nest C3 (altitude 1049.2m), air temperatures for same time period are given in broken lines. Comparison based of data from three hours datalogger recording.

1.4. Days with T>20°C, freezing days

Ants maintained a daily average temperature >20°C in their nest for a relatively short period of the year, in average 100.75 days a year. When comparing number of days with average inner nest temperature >20°C in the whole year 2010 (1st January - 31st December) differences among nests occur, reaching values from 62 to 172 days a year (figure 6). The highest number of days with T>20°C appeared in nest A1, second highest was nest B3 and than nest C3. Nest with the smallest number of days with T>20°C in whole year 2010 was nest B2, which was also smallest one. Nests A1, B2 and B3 were significantly different from all other nests ($p<0.01$), but no difference was found when comparing only localities (t-test $p>0.5$). The difference among individual nest could be explained by following multiple regression model: [day20] = 75.973 - 0.07547*[altitude] + 22.353*[year.aver]

This means that significant contribution to explained variability in number of days with T>20°C is made by altitude ($p = 0.0073$) and year average temperature of each nest ($p= 7.69e-06$). These two factors explained together 98.62% of variability found in number of days with T>20°C. Neither nest volume, nor nest moisture effect were significant, but they increased the proportion of variability explained by model to 99.66%. Shading of nest and summer sun insulation level did not play any important part in this model.

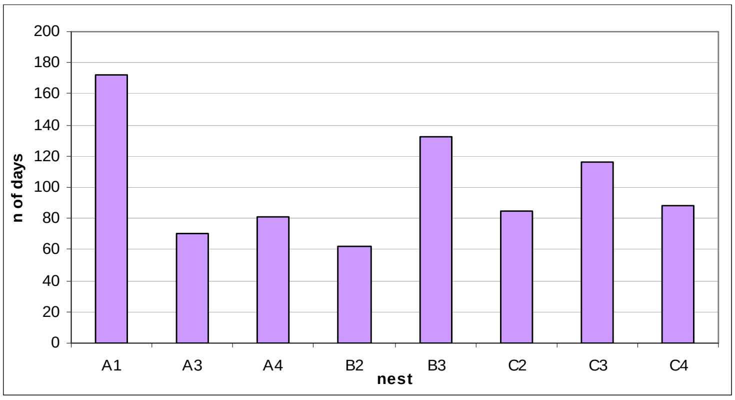


Figure 6. Number of days with average inner nest temperature T>20°C counted for whole year 2010 (n total = 365 days). Values are given only for those nests where data from both datalogger sets covering whole year 2010 were available

Comparing number of days with T>20°C in summer 2009 and 2010 (figure 7) revealed no significant difference (t-test $p>0.1$). The temperature limit of 20°C was not exceeded in all days. Extremely low number of days with T>20°C was found in nest B2, only 58.7% of all days in summer 2009 and 67.0% of all days in summer 2010. Second nest with lowest number of T>20°C was nest A3 with 80.4% and 58.2% of all days in summer 2009 and 20010 respectively. Nest A1 reached temperature >20°C in all summer days in both years. From graph it seems that in locality C the inner nest temperatures in summer 2009 are higher than in summer 2010, whereas most nests in locality A and B exhibit opposite pattern. But this difference is not statistically significant.

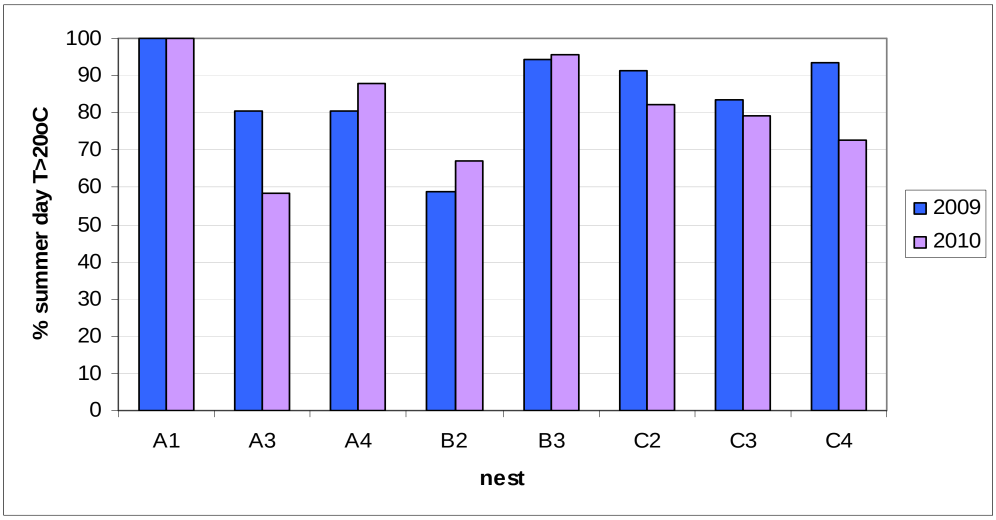


Figure 7. Number of days with average inner nest temperature >20°C in summer 2009 compared to summer 2010. Number of days is given in percent of all summer days, n total = 91.

Freezing temperatures occurred mostly in winter, but also in early spring and late autumn. Average number of days with T<0°C in whole year 2010 pooled for all nests was 62.75 with notable differences among individual nests. The highest number of inner nest temperatures T<0°C was found in nest C2, making in total 83 days and in nest B2 making in total 82 days. In contrary the smallest number of days with T<0°C occurred in nest C3, in total 49 days (figure 8). Most nest shower freezing temperatures in range 49-66% of all winter days. The lowest recorded nest temperature in winter 2009/10 was -6.1°C in nest C2 on 21.12.2009 and in winter 2010/10 it was -7.2°C again in nest C2 on 27.12.2010. Apart form nest C2 extremely low inner nest temperatures, lower than -5°C were recorded in nests C4 and A3 both in February 2011.

When focusing on factors explaining differences in number of days with T<0°C among nest we got only one statistically significant factor: nest volume (p = 0.0124). Altitude of nest is behind the limit of significance (p = 0.0705), but it also contribute to model explanatory strength, nest volume together with nest altitude explain 74.57% of variability in number of days with T<0°C. The best explanatory model is [freeze.d] = 12.665 + 0.07094*[altitude] - 28.070*[volume].

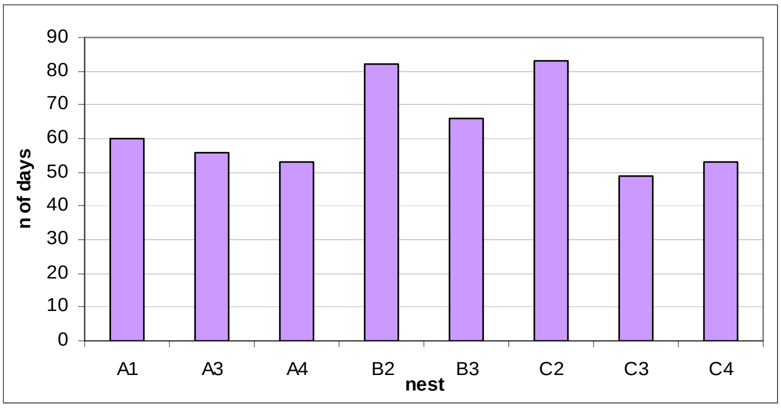


Figure 8. Number of days with average inner nest temperature T<0^{o}C counted for whole year 2010 (n total = 365 days). Values are given only for those nests where data from both datalogger sets covering whole year 2010 were available

Comparison of number of days with T<0^{o}C between winter 2009/10 and 2010/11 showed very scattered pattern, there were great differences of number of days with T<0^{o}C between the two measured years, winter 2010/11 showed lower number of freezing days, this was especially evident at locality A (figure 9). The difference was behind the limit of significance (p = 0.07). Nest A2 got never frozen in winter and spring 2011, neither in autumn 2009. Data from nest A from first measured period are missing, but we suppose there occurred freezing days same as in other nests in locality A.

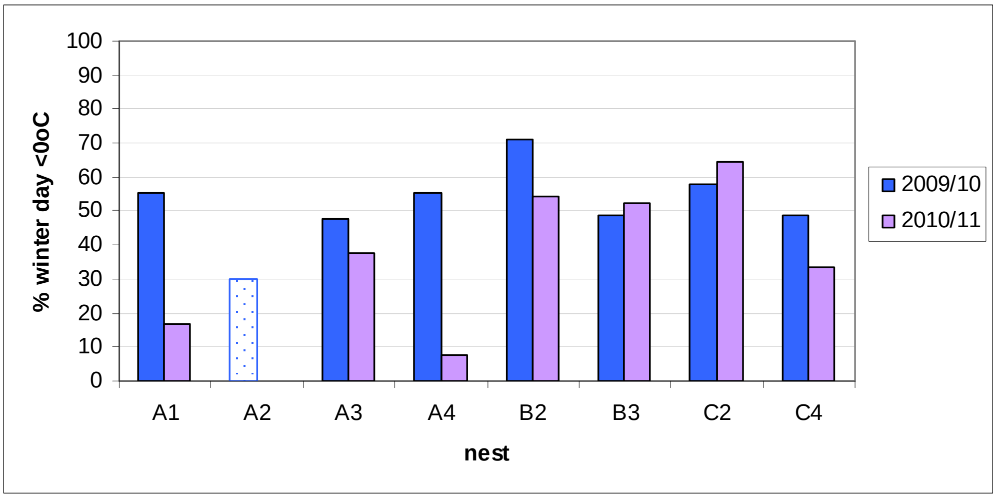


Figure 9. Number of days with average inner nest temperature <0^{o}C in winter 2009/10 compared to winter 2010/11. Number of days is given in percent of all winter days, n total = 89. Number of freezing days for nest A2 in 2009/10 is approximated from all other nest data in 2009/10 and A1 data in 2010/11.

2. Daily temperature regime

2.1. Pattern of daily temperature changes

Daily temperature regime in summer was quite universal for all nests independent of size or location (figure 10a). The highest temperature occurred in the late evening, mostly around 8AM or during night, temperature around midnight and 2-3 hours later were still high in summer. In the morning the inner nest temperature sharply dropped with minimal values reached between 8 -10 PM. In some nests the temperature decrease was shifted to midday or early afternoon (the latest minimal temperature was recorded at about 2 AM). This shift was more proposed in late summer and happened more often in bigger nests. During afternoon inner nest temperatures were increasing to evening/night maximum. Morning decrease and evening increase of individual nest inner nest temperature were of different scale and steepness, with minimal temperature change 0.3^{o}C and maximal 6^{o}C.

Daily temperature regime in autumn differed according to individual nest, date, ambient temperature or other parameters. In general three patterns were found: The most common was mild increase of inner nest temperature with minimal temperature occurring early in the morning (before sunrise) and maximal in the late evening or at night. Second pattern was a modification of summer daily temperature regime, with the lowest temperatures in the morning or around midday, the highest temperature was reached at about 8 AM, but the night temperature was lower and decreasing quickly when compared to summer pattern. Third pattern was the most common in late autumn, inner nest temperatures were more or less stable during the whole day, morning temperatures were often slightly higher than evening temperature; maximal daily fluctuations were 1-2^{o}C.

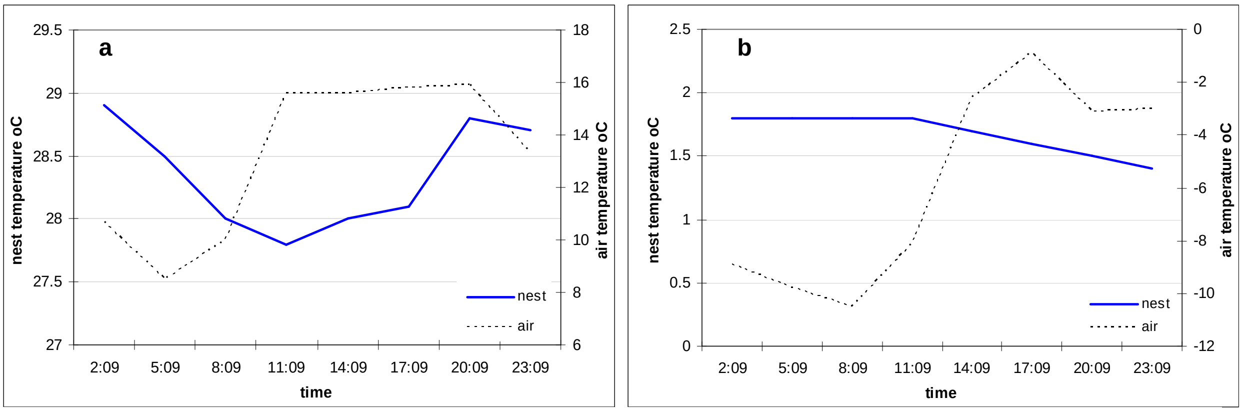


Figure 10. Typical daily pattern of inner nest temperature changes in summer (a) and winter (b), data given for nest A1, whole day dataloggers records. Note that scale of air temperature differs from nest temperature scale, nest temperature scale range is always 2.5^{o}C, air temperature scale range is 12^{o}C. Both pictured regimes concern day with no rain.

In winter there were almost no fluctuations in daily nest temperatures. Inner nest temperature was very stable often in sharp contrast to ambient temperature (figure 10b).

In early spring there the daily temperature regime was very variable, sometimes opposite patterns occurred in consecutive days. In most nests there was obvious increasing trend in daily nest temperature with temperature change between 0.5 – 1°C, or the temperature was stable. In some cases the temperature trend was decreasing, but still the general trend of temperature changes among days was increasing. Later in spring there was obvious development of summer daily pattern, with temperature decrease in the morning or around midday and high evening temperatures. In contrary, small nests were usually tightly following ambient temperature pattern with maximal temperature in the afternoon or shortly after midday.

In case of rainy weather a change of common daily temperature regime occurred in some nests. An example of summer rainy day could be seen in figure 11a. Ambient temperature was decreasing during morning to midday minimum, while the inner nest temperature peaked at this time. An increase of ambient temperature in afternoon made the inner nest temperature drop. In autumn no peculiarities occurred, inner nest temperature passively followed ambient temperature decrease, with highest temperature in morning and lowest in evening. This is a very opposite pattern of common temperature change in autumn. Also in spring rainy day the inner nest temperature decrease together with air temperature, but also common summer pattern could occur with maximal temperature late at night and minimal around midday.

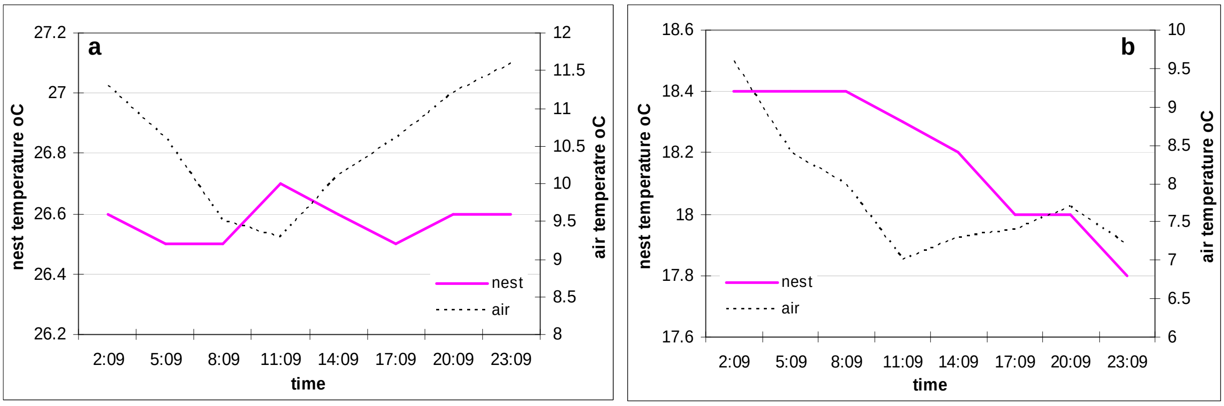


Figure 11. Daily pattern of inner nest temperature changes in rainy day - summer (a) and autumn (b), precipitation 22.5mm on 24th June and 21.0mm on 10th October, both days were preceded by at least three consecutive days of rain. Data given for nest A1, whole day dataloggers records. Note that scale of air temperature differs from nest temperature scale.

2.2. Daily temperature fluctuation

Fluctuation of nest temperature was significantly lower than air temperature fluctuation in all season and both years. In general small nests showed bigger fluctuations, but still most of nest showed similar characteristics and belong to closely related statistically homogenous groups, the biggest differences among nests temperature fluctuation appeared in summer (table 3, appendix). Air temperature fluctuation among localities A, B and C were significantly different in all seasons (Tukey HSD test, $p<0.001$). Fluctuations at locality C were nearly two times lower (mean 4.2°C a day) than at locality A and B (mean 7.3 and 8.0°C day)

In spring average fluctuations of inner nest temperature were 2.55°C a day in 2010 pooled for all nests and 2.16°C a day in 2011 respectively. The biggest fluctuation occurred on 29.3.2011 in nest C1, between 11AM and 2PM the temperature jumped form 4.5°C to 26.6°C, making the difference 22.1°C in three hours. In contrary nest A1, A2 and C4 were the most stable. (figure 12). Air temperature fluctuation was 8.38°C a day in spring 2010 and 9.66°C a day in spring 2011. Maximal air temperature fluctuations 28.6°C a day occurred on 25.4.2010 at locality A and B.

In summer inner nest temperatures fluctuations were 3.30°C a day in 2009 pooled for all nests and 2.95°C a day in 2010 respectively. Maximal nest fluctuation 18.0°C a day occurred on 18.7.2009 in nest C3. Minimal fluctuations occurred in nest B4 in 2011, with mean fluctuation 0.84°C a day, this nest created an independent group. Nest temperature fluctuations differ among individual nests especially in 2011, in 2010 there was similar pattern of nest temperature fluctuation among nests in locality C, except of nest C1. Air average fluctuation was 7.34°C a day in 2009 and 7.20°C a day in 2010. Maximal recorded air temperature fluctuation was 17.9°C a day on 25.6.2010. Ambient temperature fluctuation at localities A and B did not significantly differ from each other, but it differed from air fluctuation at locality C significantly (Tukey HSD test, $p<0.001$), the air fluctuations were same in both years.

Autumn nest temperature fluctuations were 6.02°C a day in 2009 and 5.79°C a day in 2010. Fluctuations of individual nests did not differ significantly, but some differences could be seen between locality A and C. Air temperature fluctuation was nearly three times higher than inner nest temperature fluctuation, it was 6.02°C a day and 5.80°C a day in 2009 and 2010 respectively. The difference was highly statistically significant (t-test, $p = 0.003$). Air temperature fluctuations differ significantly among localities A, B, C (Tukey HSD test, p<0.001). Maximal ambient fluctuation reached 21.8°C a day on 29.10.2010, whereas maximal nest temperature fluctuation was 11.2°C a day in nest A3 on 1.9.2009

Winter nest temperatures were extremely stable, average temperature fluctuation were 0.52°C a day in winter 2009/10 and 0.40°C a day in 2010/11, whereas the ambient temperatures fluctuations reached 3.81°C and 4.76°C a day in 2009/10 and 2010/11 respectively. Maximal air temperature fluctuation was 19.5°C a day in winter 2009/10 and 23.0°C a day in 2010/11. Nest with biggest temperature fluctuation was C2. In winter all nests in both measured years fell into one statistically homogenous groups, which means there was no difference among nest temperature fluctuation at all (Tukey HSD test, p<0.001). Air temperature fluctuations at localities A, B, C were significantly different from each other.

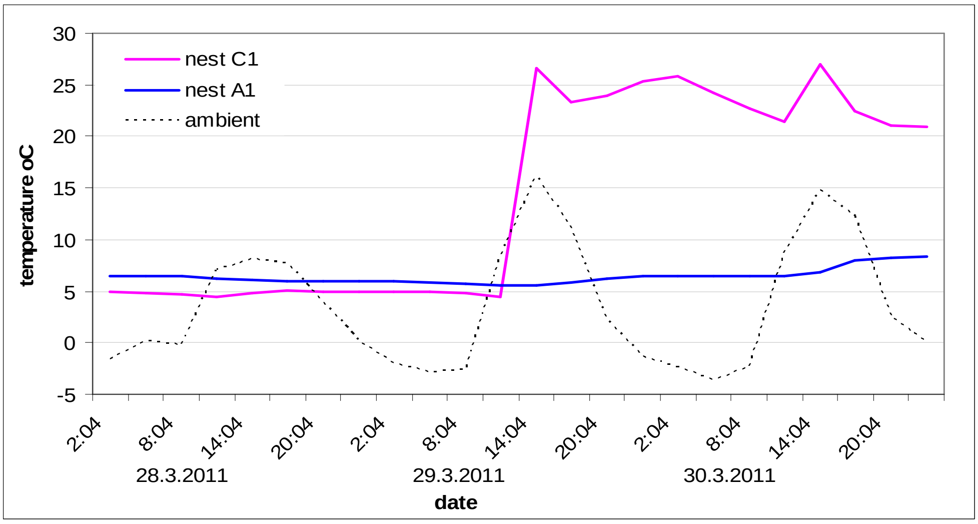


Figure 12. Fluctuations of inner nest temperature in nest C1 (maximal fluctuation found in whole measured period) and nest A1 compared to ambient temperature fluctuation, date 28.-30.3.2011.

2.4. Factors explaining fluctuations of inner nest temperature

Factors explaining night thermoregulatory behavior in ant nest were explored separately for individual seasons using linear models. Plots concerning individual seasonal models can be found in appendix (figure 4, 5). In summer and winter, i.e. periods with stable temperature plateau, the most important factor influencing fluctuation of inner nest temperature was nest identification and air temperature fluctuation. In spring and autumn, the periods were nest temperatures were changing showed a bit different effect.

In summer other factors with significant effect were mean air temperature, year, nest moisture, mean nest temperature (p<0.001), also altitude played an important role (p = 0.001).

This model explained 98.69% of all variability in summer daily fluctuation in inner nest temperature. There was nearly no unexplained variability left.

In winter other significant factors apart from nest identification and air temperature fluctuation were mean nest temperature, year, nest volume (p = 0.001). Date and mean air temperature effect were not significant, but their deletion from model caused significant change in residual sum of squares. Still this model explained only 27.53% of all variability in winter daily fluctuation in inner nest temperature. There are to be other factors playing important role in winter daily temperature regime.

Response variable: daily fluctuation of inner nest temperature in summer			
Factor	Sum Sq	F value	p
nest	1650.0	48.160	***
air temp. fluctuation	1309.3	420.376	***
mean air temperature	488.9	156.982	***
year	220.4	70.771	***
moisture	157.5	50.572	***
mean nest temperature	123.6	39.675	***
date	58.5	18.779	***
altitude	32.0	10.286	**
residuals	53.26		

Table 7 – factors explaining daily fluctuation of inner nest temperature in summer. Factors ordered according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

Response variable: daily fluctuation of inner nest temperature in winter			
Factor	Sum Sq	F value	p
nest	60.60	31.5804	***
air temp. fluctuation	26.34	137.2617	***
volume	21.71	113.1292	***
mean nest temperature	11.40	59.4132	***
year	5.82	30.3420	***
date	1.13	5.8969	*
mean air temperature	0	0.0179	ns
residuals	334.27		

Table 8 – factors explaining daily fluctuation of inner nest temperature in winter. Factors ordered according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

In spring the most important factors were mean air temperature and air temperature fluctuation, followed by nest volume, precipitation, nest identification, mean nest temperature and year, all these factors were highly significant (p = 0.001). Neither altitude nor nest moisture had significant effect onto spring daily fluctuation. This model explained 53.78% of all variability in spring daily fluctuation of inner nest temperature.

In autumn the most important factors were year and nest identification, followed by air temperature fluctuation, altitude, mean air temperature and rain. All these factors were highly significant (p = 0.001). Effect of date and mean nest temperature were not significant, but their deletion from model caused significant change in residual sum of squares. Neither nest volume nor moisture played an important role. This model explained only 38.21% of all variability in autumn daily fluctuation of inner nest temperature.

Response variable: daily fluctuation of inner nest temperature in spring			
Factor	Sum Sq	F value	p
mean air temperature	1936.4	544.2794	***
air temp. fluctuation	1139.1	320.1764	***
volume	587.4	165.1100	***
rain	519.5	1.8722	***
nest	425.7	13.2967	***
mean nest temperature	308.9	107.0527	***
year	104.4	29.3521	***
residuals	4315.4		

Table 9 – factors explaining daily fluctuation of inner nest temperature in spring. Factors ordered according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

Response variable: daily fluctuation of inner nest temperature in autumn			
Factor	Sum Sq	F value	p
year	456.20	349.7860	***
nest	355.52	27.2593	***
air temp. fluctuation	289.02	221.6045	***
altitude	55.62	42.6481	***
mean air temperature	26.21	20.0978	***
rain	24.74	18.9709	***
date	3.94	3.0186	ns
mean nest temperature	0.01	0.0045	ns
residuals	1958.95		

Table 10 – factors explaining daily fluctuation of inner nest temperature in autumn. Factors ordered according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

2.5. Night temperature change

To observe nest thermoregulatory behavior independent of insulation we compared night temperature change between 12PM and 3AM. Concerning whole year data night temperature change among nest at locality A (mean -0.33^{o}C a night) was bigger than in localities B and C (mean -0.18 and -0.2^{o}C a night). In winter the most common pattern of night temperature change was no change at all, the slope 0.00^{o}C which occurred in 890 cases, which means 50.6% of all. Temperature change category from -0.4 to 0.0 ^{o}C made the great majority (figure 13), 1291 of 1759 cases. Thus we can say that during winter temperature inside nest was changing only minimally in night hours.

In spring the night temperature change between 12PM and 3AM showed normal Gaussian distribution, with the most common slope -0.4 to 0.0^{o}C. Changes bigger than -1^{o}C or +1^{o}C creates 5.6% and 0.5% respectively. Negative slopes (55.5%) were more common than positive one (21.9%), which means that the night temperature inside nests dropped more often than rise. Maximal changes in spring were -5.2^{o}C and +3.3^{o}C.

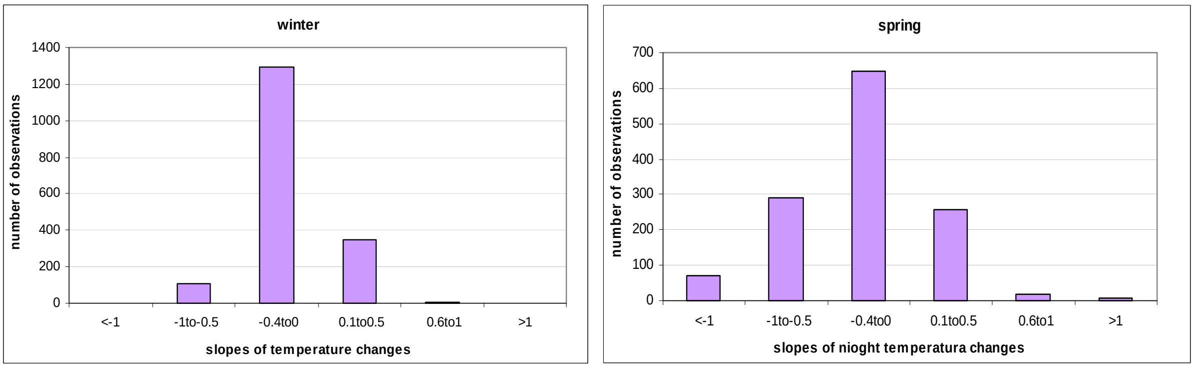


Figure 13. Slope of night nest temperature changes between 12PM and 3AM in winter (first figure) and spring (second figure), data pooled for all nests.

In summer there was marked higher proportion of negative slopes (74.8%) indicating decrease of inner nest temperature during night (1295 cases of 1730) than positive slopes (18.7% ie. 324 cases of 1730). Slopes with level 0.0 indicating no temperature change in night temperature occurred only in 6.5% cases. We can say that during summer nest temperature usually drop in range of -1 to -0.1^{o}C. The biggest change was -11.1^{o}C and +6.0^{o}C.

During autumn the most common night temperature change ranged from -0.4 to 0.0^{o}C (figure 14), but still negative slopes (53.5%) were more common than positive (25.1%).

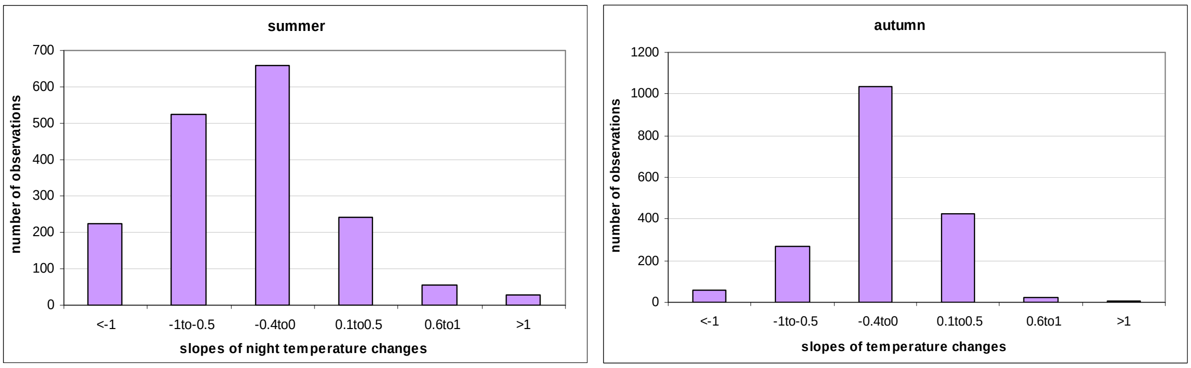


Figure 14. Slope of night nest temperature changes between 12AM and 3PM in summer (first figure) and autumn (second figure), data pooled for all nests.

2.6. Factors influencing night temperature change

Factors explaining night thermoregulatory behavior in ant nest were explored separately for individual seasons using linear models. Plots concerning individual seasonal models can be found in appendix (figure 6, 7). In all seasons the most important factor influencing night temperature change was nest identification, this is not surprising because the nest share all characteristics such as nest volume, moisture, altitude, shading and insulation level. But still there is lot of variability left to be explained by other factors. Other factors such as air or nest temperature and fluctuation and precipitation were unique for each day.

In summer the most important factor influencing night temperature change just after nest identification was inner nest temperature fluctuation, followed by mean air temperature and air temperature fluctuation. Effect of mean nest temperature was not significant, but its deletion from model caused significant change in residual sum of squares. Other significant factors were nest moisture, date and year, effect of altitude was significant on p=0.01. This model explained 27.18% of all variability in summer night temperature change.

In winter the most important factor just after nest identification was air temperature fluctuation, followed by mean nest temperature, year and altitude, all these factors were highly significant with p<0.001. Date and mean air temperature effect were not significant, but their deletion from model caused significant change in residual sum of squares. Neither nest volume, nor nest moisture effect played an important role. This model explained 28.40% of all variability in winter night temperature change.

Response variable: Summer night T. change			
Factor	Sum Sq	F value	p
nest	162.58	26.9787	***
nest temp. fluctuation	153.43	279.9871	***
mean air temperature	13.16	24.0174	***
air temp. fluctuation	8.41	15.3559	***
moisture	4.12	7.5109	**
date	3.27	5.9722	*
altitude	2.40	4.3776	*
year	2.17	3.9581	*
mean nest temperature	0	0.0020	ns
residuals	936.50		

Table 11 – factors explaining night temperature change of inner nest temperature in summer. Factors ordered according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

Response variable: Winter night T. change			
Factor	Sum Sq	F value	p
nest	84.53	39.5780	***
air temp. fluctuation	24.41	125.7233	***
mean nest temperature	12.37	63.6982	***
year	3.31	17.0535	***
altitude	2.24	11.5157	***
date	0.29	1.4799	ns
mean air temperature	0	0.0005	ns
residuals	320.56		

Table 12 – factors explaining night temperature change of inner nest temperature in winter. Factors ordered according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

In spring the most important factor just after the nest identification was nest temperature fluctuation (p<0.001), other significant factors were mean air temperature, altitude and date (p<0.001); air temperature fluctuation and nest volume (p=0.001) and mean nest temperature and precipitation (p=0.01). This model explained 21.82% of all variability in spring night temperature change.

In autumn most important factor just after the nest identification was year (p<0.001), followed by air temperature fluctuation, altitude, date, mean nest temperature (p<0.001) and precipitation (p=0.01). Neither nest volume nor nest material moisture effect were significant. This model explained 21.61% of all variability in autumn night temperature change.

Apart from nest identification effect there were 4 factors playing significant role in explanation of night nest temperature change in all seasons: the date, air temperature fluctuation and altitude. Mean nest temperature occurred in all cases, but in summer its effect was not significant. Rain played an important role only in spring and autumn, the seasons were inner nest temperature was changing. Volume or moisture were significant in one of four seasons only; volume in spring and moisture in summer night temperature change. Level of nest shading was never significant.

Response variable: Spring night T. change

Factor	Sum Sq	F value	p
nest	29.13	13.8209	***
nest temp. fluctuation	14.43	61.6360	***
mean air temperature	5.63	24.0532	***
altitude	4.54	19.4046	***
date	3.23	13.7919	***
air temp. fluctuation	2.48	10.5849	**
volume	1.90	8.1087	**
mean nest temperature	1.48	6.3244	*
rain	1.11	4.7440	*
residuals	229.03		

Table 13 – factors explaining night temperature change of inner nest temperature in spring. Factors ordered according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

Response variable: Autumn night T. change

Factor	Sum Sq	F value	p
nest	54.97	28.6241	***
year	7.32	38.0950	***
air temp. fluctuation	6.90	35.9325	***
altitude	3.86	20.1197	***
date	3.17	16.5107	***
mean nest temperature	2.30	11.9586	***
rain	1.06	5.5039	*
residuals	288.66		

Table 14 – factors explaining night temperature change of inner nest temperature in autumn. Factors ordered according to explained sum of squares. Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’

3. Detailed measurement of inner nest temperature

3.1. Averages of inner nest temperature in depth 15, 10, 5cm

In 2010, during period of increased ant activity, a detail measurement of inner nest temperature in three different depths (15, 10, 5cm) bellow hill top was conducted by method of spot sampling by hand. These data should reveal the pattern of thermal flow in the nest. Sampling was repeated five times a day (for details see methodic). In general average temperature was increasing from 5 to 15cm depth below nest surface; this difference was not significant although often on border of 0.05 significance level.

There was found a significant effect of locality (one-way ANOVA, Tukey test, p<0.001) onto average inner nest temperature I all measured depths, but it explained only 9.28% of all variability. Thus we are not surprised by the fact, that not all nests from the very same locality (A, B, C) always showed identical characteristics concerning the inner nest temperature in three different depths (15, 10 and 5cm below hill top). The biggest variability of average nest temperature in all measured depths can be seen among nests in locality A; in contrary nests from locality B were similar to each other, they belong to same or at least similar statistically homogenous groups (table 15). Similar characteristics could be found among nests with big nest volume (and presumably big population number), namely nests C3, C4, A2.

nest	**inner nest temperature (°C)**											
	depth 15 cm			**depth 10 cm**			**depth 5 cm**			**difference 10-5 cm**		
	mean	SD		mean	SD		mean	SD	group	mean	SD	
A1	23.70	5.77	efg	22.73	5.41	ad	20.43	5.10	de	2.30	1.26	c
A2	25.78	4.84	g	25.62	4.86	e	24.71	4.99	g	0.91	1.01	a
A3	21.48	5.74	cde	20.69	5.77	ab	18.45	5.38	bcd	2.25	1.66	c
A4	21.05	5.89	bcd	20.66	5.55	ab	19.43	5.31	cde	1.22	1.42	ab
B1	19.84	5.41	abc	19.17	5.18	bc	17.92	5.23	abc	1.25	1.29	ab
B2	19.04	6.81	ab	18.43	6.50	bc	17.07	6.20	ab	1.36	1.34	abc
B3	22.13	6.23	cde	20.59	6.24	ab	18.79	5.98	bcd	1.80	1.35	abc
B4	18.38	3.94	a	17.42	4.10	c	16.01	4.22	a	1.40	0.66	abc
C1	22.79	6.36	def	21.92	5.90	a	19.90	5.72	cde	2.02	1.25	bc
C2	22.97	5.48	def	22.47	5.70	ad	21.09	5.50	ef	1.37	2.33	abc
C3	24.79	6.14	fg	24.24	6.18	de	23.05	6.37	fg	1.20	0.83	ab
C4	25.80	5.12	g	25.56	5.35	e	24.65	5.80	g	0.91	0.75	a

Table 15. Average values of inner nest temperature for all sampled ant nests. Data from whole spot sampling period pooled. Temperature is given in °C, statistically homogenous groups are marked by the same letter (ANOVA; Tukey test; $P < 0.001$).

By comparing results for each nest among localities, an interesting pattern of average nest temperature in all three measured depths was found. Nests from locality A do not significantly differ from nests from locality C (t-test, p>0.4), however between nests from locality B and C there is highly significant difference (p<0.01 in all measured depths). A difference also occurred between nests from locality A and B, although they differ in altitude only little, this difference was above border of significance p=0.05 for depth 5 and 15cm, and below this border for nest depth 10cm (p=0.039).

Focusing on factors that affect nest temperature in three different depths (5, 10, 15cm below hill top) the biggest proportion of variability is explained by the date of study and nest identification, both these factors were highly significant (table 16). Important factor was also time of day when the sampling was done. This model explained x% of all variability. There was no significant influence of insulation level, average air temperature, nest size or nest material moisture.

factor	Nest temperature in **15cm**			Nest temperature in **10cm**			Nest temperature in **5cm**		
	SS	F	p	SS	F	p	SS	F	p
nest	215	24.7	<0.001	245	29.2	<0.001	278	35	<0.001
date	1669	191.8	<0.001	1616	192	<0.001	1569	198	<0.001
time	30	3.4	0.009	29	3.4	0.009	47	5.9	<0.001

Table 16. Analysis of factors that explain nest temperatures in three different depths (5, 10, 15cm below hill top) using General linear models. Only significant correlation coefficients are given.

Concerning the temperature difference between 10-5cm, all nest show similar characteristics. The difference among localities A, B or C was not significant. The temperature difference between the mound depth 10 and 5 cm was in average positive, the negative values counted for 10% from all cases (figure 15). This difference is directly proportional to the heat flow in the nest; which indicates that the heat flows from inside out.

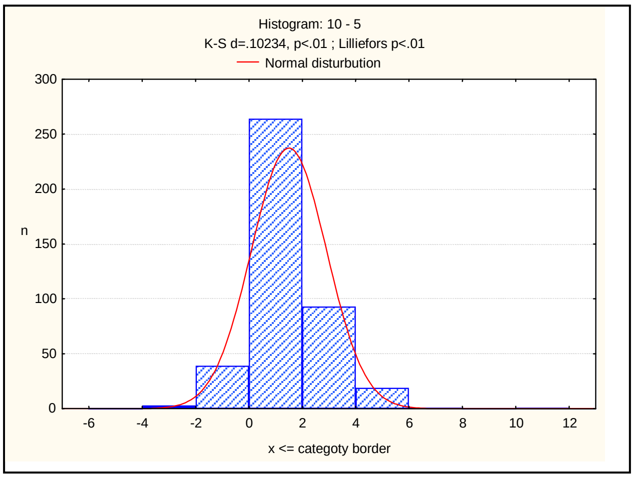


Fig. 15. Distribution of temperature difference between depths 5-10 cm in *F. polyctena* nest (pooled for all nests) compared to normal distribution

Inner nest temperatures were not the same through all day. Similar pattern of daily temperature changes could be seen among all nests. In the morning inner nest temperatures were low, than slowly heating of nest was evident, but the temperature did not peak at midday (figure 16), although at this time the highest air temperature and biggest income of solar energy occurred. The maximal inner nest temperature (depth 15cm) was reached in the evening one hour before the sunset. After the sunset, during night the inner nest temperatures (depth 15cm) were decreasing, the lowest temperature was reached one hour before the sunrise. Temperatures at different day time were significantly different (table 17).

Concerning the temperature difference between 10 and 5cm depth another daily pattern occurred. The temperature difference in all day times except of midday was similar with average temperature difference 1.6^{o}C. In the middle of day the smallest difference in nest temperatures between 10-5cm below nest surface occurred, only 0.9^{o}C (figure 16).

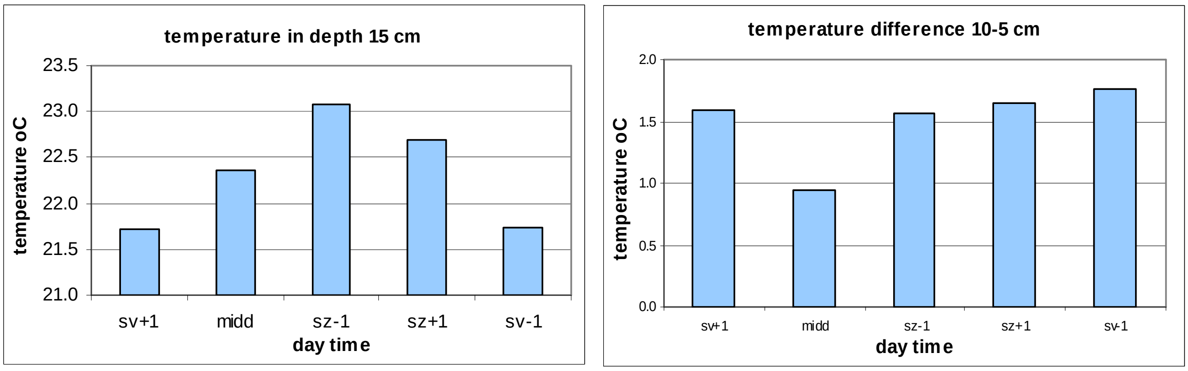


Figure 16. Average inner nest temperatures in depth 15cm and average difference in inner nest temperatures between depth 10-5cm (pooled for all nests and months) for five sampling times a day. Legend of abbreviation “s.rise+1” for one hour after sunrise, “midd” for middle of the day (note it doesn’t mean the noon), “s.set-1” for one hour before sunset, “s.set+1” for one hour after sunset, “s.rise-1” for one hour before sunrise..

time	**inner nest temperature (^{o}C)**											
	depth 15 cm			**depth 10 cm**			**depth 5 cm**			**difference 10-5 cm**		
	mean	SD		mean	SD		mean	SD		mean	SD	
s.rise+1	21.71	0.661	a	20.86	0.669	a	19.26	0.314	a	1.598	0.143	b
midd	22.36	0.677	ab	21.85	0.665	ab	20.91	0.310	b	0.938	0.149	a
s.set-1	23.07	0.683	b	22.27	0.672	b	20.71	0.311	b	1.562	0.146	b
s.set+1	22.69	0.679	ab	21.97	0.669	ab	20.32	0.313	ab	1.646	0.171	b
s.rise-1	21.74	0.668	a	21.17	0.682	ab	19.42	0.312	a	1.755	0.148	b

Table 17. Average nest temperature (pooled for all nests) in different depths (5, 10,15 cm below hill top) for five sampling times in a day. Data from spot sampling by hand covering time period from end of April 2010 to end of September 2010. Temperature is given in °C, statistically homogenous groups are marked by the same letter (ANOVA; Tukey test; $P < 0.05$).

3.2. Day regime of inner nest temperature - comparison of data obtained by 10-hour spot sampling and dataloger records

Continuous 12-hour spot sampling was carried out in rainy conditions, it started after midday and continued overnight till sunrise of next day. Data from spot sampling correspond well with data obtained from dataloggers (see chapter 2.1.). In all nest except for nest C1 the core temperature was in between the range given by temperatures records from depth 5, 10, 15cm below nest surface (figure 17). The average nest temperature usually increased from 5cm to 15cm layer, nest temperature in 5cm fluctuated more than temperature in deeper layers or in nest core. Temperatures on nest surface corresponded tightly with the ambient temperature in all nests. Ambient and nest surface temperature was highest in the afternoon, between 3 and 6AM. From evening to morning air temperature was decreasing in all localities, at locality A and B it was 4^{o}C in average, at locality C it was 6^{o}C. There was a remarkable difference between the surface temperature and the temperatures inside nest, even though only 5cm deep.

Most nests at localities A and C showed similar pattern of daily temperature changes, maximal inner nest temperatures in all depths were reached in late evening or close to midnight, since than inner nest temperature sharply decreased both in nest core and sampled layers. Nest C4 showed different pattern, with highest temperature in midday and decreasing temperature through evening, night till morning. In nests A1 and A2 the inner nest temperature at all depths was stable, just slightly increasing during all sampled periods, there occurred no morning temperature drop. Nest C3 shoved the most rapid decrease of inner nest temperature in morning hours (figure 17). On the other hand, nest with the most stable temperature regime was nest A2, it has also the highest core temperature (figure 18).

At locality B there was evident gentle increasing trend with lowest temperatures around midday and highest in the morning. Nest B2, which is the smallest one, showed very opposite temperature regime to other nests. Inner nest temperatures were highest at midday and sharply decreasing, more than 5^{o}C in one day, to the morning, both in nest core and all sampled layers. The temperature regime obviously copied the air temperature pattern. Nest surface temperature peaked at 15:30, apparently corresponding to maximal sun radiation income.

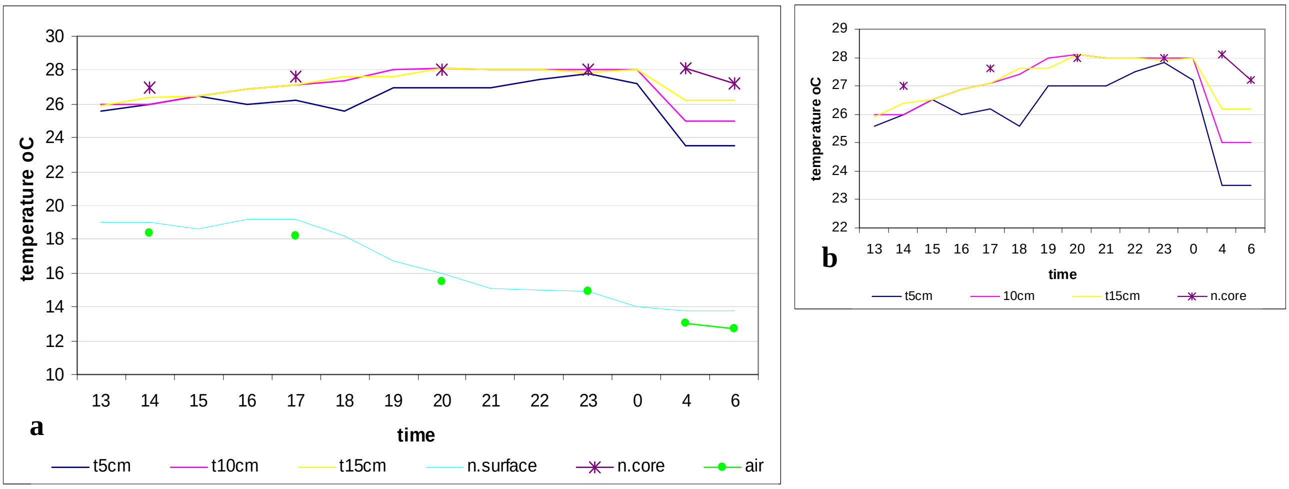


Figure 17. Daily temperature changes in nest C3, together with air and nest surface temperature (a) and detailed look onto nest temperature in different depths below hill top and in nest core (b)

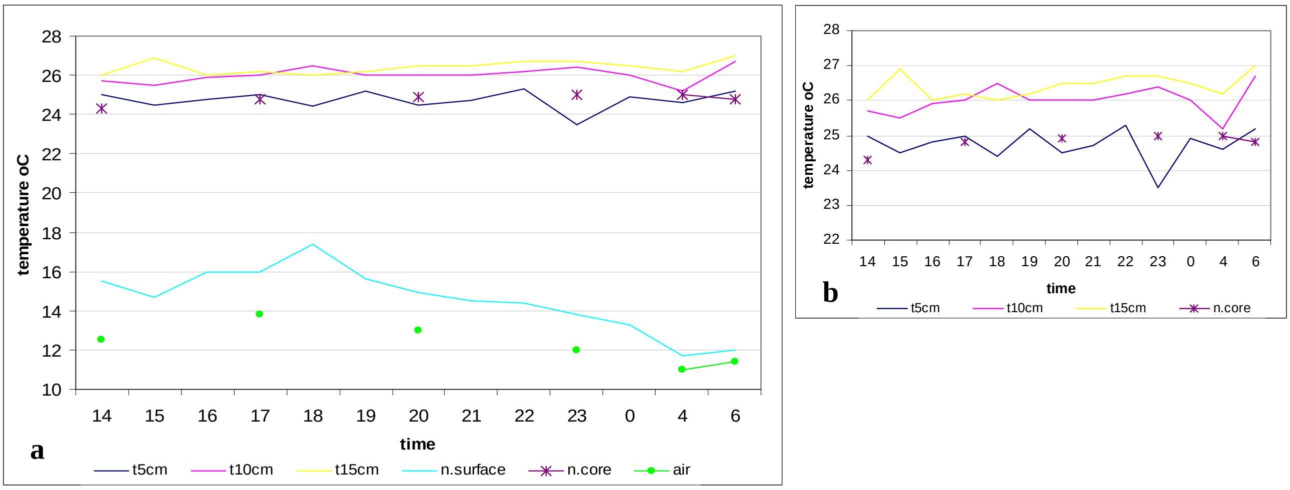


Figure 18. Daily temperature changes in nest A2, together with air and nest surface temperature (a) and detailed look onto nest temperature in different depths below hill top and in nest core (b)

3.3. Different depth temperature changes through out summer season

Next we focused on inner nest temperature in different depths and effect of month when sampling was carried out. Consecutive months do not show similar temperatures. May and June are significantly different from all other months and from each other too in all sampled depths. In June we could observe the highest inner nest temperature in all sampled layers, while May temperatures are quite low, even lower than April temperatures. April and July have same characteristic concerning temperature in depth 15 and 10cm bellow hill top, the average temperature is second highest for all sampled period. Concerning temperature in most surface layer, 5cm below hill top, similar characteristic could be seen in July and August.

In September we can always see a totally different temperature regime, it is a separate category. September inner nest temperatures were the lowest in whole sampled period, more then two times lower than in other months. This difference was highly significant. Surprisingly temperature in August 2009 and 2010 do not share the same characteristics, there is no “shoda” concerning temperature in 15cm below nest surface, August 2009 match with June 2010. Concerning temperatures in depth 10 and 5cm August 2009 and 2010 do not match, but they are close to each other (table 18).

The difference of temperature between 10 and 5cm depth displays an opposite pattern. Temperature difference is the same for all sampled months, for both years, except of April which shows significantly higher difference in inner nest temperature between 10 and 5cm depth and belongs to a separate group.

date	**inner nest temperature (°C)**											
	depth 15 cm			**depth 10 cm**			**depth 5 cm**			**difference 10-5 cm**		
	mean	SD		mean	SD		mean	SD		mean	SD	
Apr	24.62	0.61	ab	23.96	0.62	ab	21.50	0.62	c	2.465	0.251	b
May	21.84	0.86	c	20.82	0.86	d	19.29	0.86	b	1.533	0.201	a
June	25.39	0.40	a	24.75	0.43	b	23.23	0.43	d	1.517	0.227	a
July	24.53	0.36	ab	23.85	0.36	ab	22.58	0.36	cd	1.272	0.101	a
Aug	23.63	0.35	b	23.15	0.35	a	22.06	0.35	cd	1.097	0.146	a
Sep	10.69	0.27	d	10.24	0.26	c	8.94	0.26	a	1.318	0.142	a
Aug 09	25.49	0.28	a	24.60	0.33	ab	23.28	0.33	d	1.297	0.091	a

Table 18. Average month nest temperature (pooled for all nests) in different depths (5, 10,15 cm below hill top). Data from spot sampling by hand covering time period from end of April 2010 to end of September 2010. Temperature is given in °C, statistically homogenous groups are marked by the same letter (ANOVA; Tukey test; $P < 0.05$).

3.4. Ant activity

Ant activity, counted as number of forages on the trail and number of nest openings (for details see chapter methodic) was measured during spot sampling. According to ordinate diagram (figure 19) the ant activity is obviously strongly affected by surface temperature, which tightly corresponds to air temperature and sun radiation income (Monte Carlo test, p=0.002). Number of ants going out follows the surface temperature perfectly. Number of ants going inside nest was shifted towards altitude axis. The main axis, which correlates positively with total umber of ants walking, is composed of effect of surface temperature, insulation and altitude.

The opposite axis direction corresponds well with number of nest openings. This means that surface temperature (together with insulation level) correlates negatively with number of nest openings, higher surface temperature causes smaller number of nest openings. Effect of moisture goes in similar direction with number of nest openings.

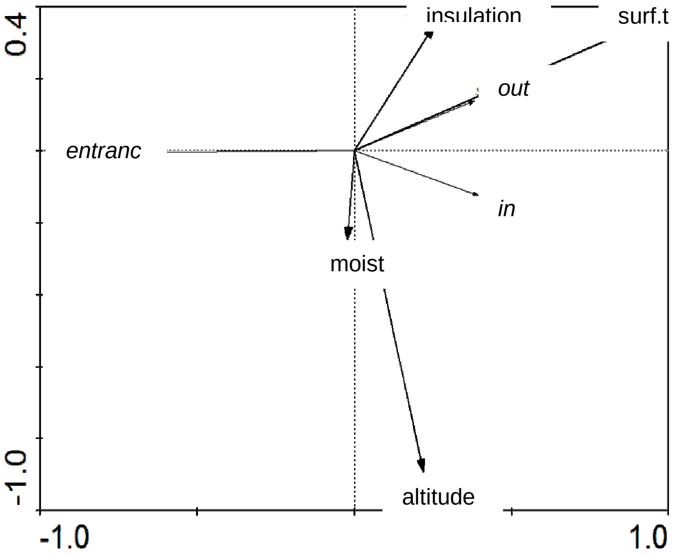


Figure 19 - Ordinate diagram (RDA) describing the dependence between ant activity: number of ants walking out/in the nest (out, in) and number of nest openings (entranc), axis showing significant factors according to table 5: surface temperature, altitude, insulation [Lux] and nest material moisture. Significance of first canonical axis p= 0.002

Focusing on factors that effect ant activity in summer period (RDA, forward selection), we found significant effect of nest surface temperature, altitude, insulation and nest moisture (table 19). The biggest proportion of variability was explained by surface temperature, which explain 22% of data variability, from other variables only altitude explained 3% of data variability, other variables explain less that 1% of data variability Neither nest volume, temperature of 5 and 15cm depth nor precipitation level had any significant effect onto ant activity.

Variable	explained variability	p	F
surface temp.	0.22	**0.002**	117.18
altitude	0.03	**0.002**	15.43
insulation	0.01	**0.004**	5.71
moisture	0	**0.044**	3.05
volume	0	0.334	1.07
temp.5cm	0.01	0.342	1.13
temp.15cm	0	0.11	2.28
rainfall	0	0.56	0.55

Table 19. Analysis of environment parameters that explain ant activity (number of ants walking and number nest openings). RDA, forward selection. Significant effect marked by boldface

V. DISCUSSION

1. Whole year temperature regime, slopes of temperature change

Average inner nest temperature in nest of *Formica rufa* ants was significantly higher than ambient temperature in all measured seasons and both years (table 1 results). This is with agreement with majority of authors from Steiner (1924), Kneitz (1964), Rosengren & col. (1987) to Frouz & Finer (2007). In summer the inner nest temperature was more than two times the air temperature, also Rosengren & col. (1987) and Hölldobler & Wilson (1990) reported very high summer nest temperatures, nearly 30°C.

High temperature inside ant nest is needed for successful brood development (Kneitz 1966, Coenen-Strass 1985, Rosengren & col. 1987), preferred temperature for pupae development in *Formica rufa* is 29°C (Coenen-Strass 1985). The most important in ant colony is production of sexual brood, because the population cannot increase its fitness through production of sterile workers only. Nests producing sexual offspring always have higher temperatures than those producing only workers. This difference persists even after the sexual offspring have left the nest (Rosengren & col. 1987). The temperature can also affect the male: female ratio of sexuals. Grösswald & Bier (1957) reported that at temperature between 13-19°C unfertilized eggs are laid, later hatching in males only. Eggs which develop at temperature higher than 19.5°C will hatch as females (Grösswald & Bier 1957 in Hölldobler & Wilson 1990). Thus small nests with worse thermoregulation could be assumed to produce more male biased brood.

However, some studies show different results. Recent study of Tuzzolino & Brown (2010) on the population ecology of a North American ant *Formica fusca* revealed that neither nest temperature nor moisture level was significantly correlated with brood production. Maybe we should consider this study an exception confirming the rule.

High temperature also speeds up all metabolic processes including the development rate (Porter 1988). Faster and shorter development is advantageous for ant community both in sense of workers production, which may facilitate production of an extra generation of workers and, thus increase the competitive power of the colony; and sexuals production, which gives new queens more time to establish and develop new colonies (Frouz & Finer 2007). High inner nest temperature is also required for queen egg lying (Kipyatkov & Schederova 1990, Frouz & Finner 2007).

Whole year temperature regime was as follow: in spring a steep increase of inner nest temperature occurred, in summer the nest temperatures were high and stable, with temperature plateau near 25°C, during autumn inner nest temperatures were decreasing to a winter plateau with temperatures near 0^oC. Spring and autumn slopes were significantly different from slope of air temperature changes. The very same pattern of year temperature changes was described in study of Frouz & Finer (2007) investigating thermoregulation of wood ants on south-north gradient.

In spring both air and nest temperature changes slopes were increasing, the slope of inner nest temperature was significantly steeper and nearly two times higher than slope of air temperature changes. In summer slopes of inner nest temperature were identical to slopes of air temperature, both of them were in general stable, yet there occurred some differences of slope direction between two measured years. In 2009 we recorded slightly increasing trends both for nests and air temperature, but in 2010 the slope of inner nest temperature was slightly decreasing in most of nests (table 2 in appendix). Slope of air temperatures in summer 2010 was stable or slightly decreasing too.

In autumn both air and nest temperature changes slopes were decreasing, the slope of nest temperature changes was significantly steeper and nearly two times lower than slope of air temperature changes. Winter slopes of inner nest temperature were identical to slopes of air temperature, both of them were in general stable close to zero, although slope of nest temperature changes was shifted towards negative values while slope of air temperature changes was slightly positive (table 2 in appendix).

Study from Frouz & Finer (2007) showed nearly identical patterns in whole year temperature changes. In summer, temperatures showed decreasing trends in most nests which means that early summer nest temperatures were higher than in late summer. Winter trends of nest and air temperature change were very homogeneous. There were observed decreasing trends in Finland, while in the Czech Republic the slopes were different between two consecutive years; in winter 2003 increasing trends occurred but in 2004 temperature slope was decreasing (Frouz & Finer 2007). To sum up, both studies found similar tends in whole year temperature pattern, agreement with Frouz & Finer (2007) occur in founding that there could appear significant differences in nest temperature between two consecutive years.

Important factor concerning thermoregulation behavior is estimation by date, or at least part of year by ant workers. The ants distinguish the date (part of the year) according to the length of light cycle; it is not determined by ambient temperature (Hölldobler & Wilson 1990). Ants start to thermoregulate early in spring, when the ambient temperatures are quite low (often still freezing), which means that energy for heating the nest is big. But in autumn, when the ambient temperatures are relatively high, ie. thermoregulation will have low costs, the inner nest temperature drops quickly, even faster than air temperature (Rosengren & col. 1987, Frouz & Finer 2007, present study).

Steep spring slope of nest temperature changes could be explained by colony needs in sense of queen oviposition and brood development, which both require high temperatures (Kipyatkov & Schederova 1990, Kneitz 1966, Coenen-Strass 1985, Rosengren & col. 1987, Frouz & Finer 2007). Queens lay eggs from early spring to mid-summer, the length of oviposition period is stable, approximately 100 days, and is probably driven by endogenous facors. Under laboratory conditions the queen lays eggs independently on the light and temperature conditions. The timing of oviposition is stable and after 68-106 days of egg laying the queen enters the diapauses (Kipyatkov & Schederova 1990). So we can assume that workers facilitate queen egg lying by maintaining high inner temperature. This idea is supported by founding of Risch & col. (2005), who reported that ant activity has the largest influence on temperature maintenance in spring and early summer.

The mechanism of steep spring slope of nest temperature changes could be explained by combination of all theories concerning thermoregulation in ant nest (see introduction). In spring solar radiation play an important role as a trigger of nest thermoregulation. First role of solar radiation is direct heating of nest material (Seeley & Heinrich 1981, Frouz 2000, Penick & Tschinkel 2008), and second heating ant bodies while basking in the sun on nest surface (Zahn 1958). Ant bodies are great energy collectors (Frouz 1996), accepted heat is later brought into the mound, where it helps to heat nest interior. This behavior could be typically observed in spring (Rosengren & col. 1987, Frouz 2000). A cumulative positive feedback of temperature increase was found in ant nest. It is assumed that first little temperature increase caused either by sun radiation (direct effect of insulation or heat captured during ant sunning behavior) or ant metabolic heat released from fatty reserves of young workers (Martin 1980) cause further increase of microorganism activity (Coenen-Stass & col. 1980; Frouz & col. 1997) and stimulate other workers to wake up and work, which results in massive heat increase in short time period.

Also explanation of autumn temperature drop corresponds with ant reproduction. In late summer the queen stopped lying eggs and entered diapauses (Kipyatkov & Schederova 1990), in autumn there is no more brood present in the nest, thus thermoregulation is not needed. Moreover lower temperatures are advantageous for workers because of prolonged life expectances (Porter 1988, Porter & Tschinkel 1993). Later in autumn ants start to move to underground chambers. (Hölldobler & Wilson 1990, Rosenren & col. 1987) Effect of sunny days could not increase temperature in nest without ants, because both important heat source and a heat storage capacity (Frouz 2000) are already missing.

Thermoregulation behavior described above is universal for wood ants. According to Frouz & Finer (2007) that geographic differences seem to be less important than variation between seasons, which indicates that maintenance of internal nest temperature is important part of wood ants' biology.

2. Seasonal inner nest temperature averages

Average temperatures of nest were higher than ambient temperatures in all seasons. There was found a significant difference both among measured nests and between two years of study. Seasonal average nest temperatures among localities A, B, C were not significantly different, except of winter 2010/11 when a significant difference between localities A and B was found. This is in agreement with study of temperature regime along south-north gradient (Frouz & Finer 2007), which reported that differences in nest temperature between Finland and the Czech Republic were not bigger than differences between two consecutive years in the Czech Republic.

Whole year datalogger records revealed significant differences in nests average temperatures among all measured nests, while air average temperature at localities A, B, C did not differ significantly. This is quite surprising for general ecological studies usually proclaim that with every 100m of altitude increase there is a temperature drop (Begon & col. 1990). Previous study in National Park Šumava (Vojtěch pers.com) showed 1^{o}C temperature drop for each 100m of altitude increase, this values were obtained from measuring temperature in well connected spruce forest on north-west hill slopes.

In spring 2010 the average nest temperature was 10.8^{o}C, while pooled March and April average nest temperature in 2011 was only 5.4^{o}C. In other words, May temperatures are the highest in all spring, while in March and April the inner nest temperature is similar to ambient temperature. This is in agreement with presented data concerning spring temperature increase, which usually occurs in the second half of April (see next part). These data also suggest great importance of May inner nest temperature for colony life, which corresponds to Risch & col. (2005) founding that ant activity has the largest influence on thermal homeostasis in spring and early summer.

From May onwards a summer temperature plateau at about 25^{o}C was reached, nest temperature was too times higher than air temperature. Nest at Zelená hora Mountain showed the most homogenous temperatures of all. On contrary nests at locality A, the lowest one, showed very variable pattern of inner nest temperature. We can speculate about selection pressure onto life in higher mountains with ideal temperature selected, whereas in lower locations the temperature conditions may be favorable enough to allow also survival of nests with less appropriate thermoregulation.

Maximal and minimal nest temperatures recorded in summer was 31.6^{o}C and 6.8^{o}C respectively. Study of Frouz & Finer 2007 reported similar summer temperatures with maximal fluctuation between 12.1 to 32.0^{o}C. Minimal temperature varies between these two studies, but we can observe certain maximal temperature limit, in this case 32^{o}C, which is precisely guarded not to be exceeded. Similar pattern occurs in *Apis melifera*, where temperature higher than 36^{o}C is strongly avoided, because they cause malformations in development and bigger brood mortality (Seeley & Heinrich 1981). The lethal temperature for workers F.polyctena is 40^{o}C (John 2008).

In autumn a slowly decreasing pattern was evident through all months. In 2009 a sharp drop was observed in October, temperatures dropped 15-20^{o}C in a single week. From this day onwards the nest temperatures stayed low. In 2010 the temperature decrease was milder. The long-term experiments of Kipyatkov & Schederova (1985, 1990) indicated regular alternation in queen reproduction and diapauses. The duration of queen egg laying period is 62-107 days, after this time the queen enters diapauses. This may explain why the temperature in their nest suddenly decreased after a certain time even when ambient conditions did not change dramatically (Frouz & Finer 2007).

In winter a thermal plateau of inner nest temperature was reached with nest average temperature 0.41^{o}C pooled for all nest and both season. Nest temperature was higher than ambient temperature; there was a marked difference in air temperature between two measured years. In 2009/10 all three localities A, B, C showed identical winter air temperature, in 2010/11 there was difference among all localities. On the contrary, inner nest temperatures were similar in all measured nest and both years.

Maximal and minimal recorded winter nest temperature recorded was +8.7^{o}C and -7.2^{o}C respectively. Study from Frouz and Finer (2007) reported winter fluctuation of inner nest temperature –6.8 to +5.1^{o}C. We can say that winter fluctuation of inner nest temperature were slightly higher in Šumava mountains. According to Frouz & Finer (2007) minimum nest temperature was reached in February – March. This study recorded the lowest temperatures in February in both years, but in some nests very cold temperatures occurred also in December 2010 (C2), January 2011 (B2) or March 2011 (C4).

Study of Frouz & Finer (2007) was focused only on factors affecting nest temperature during summer. Among significant factors was founded a combination of location and year of study, with degree of shading explained the largest proportion of variability in daily average nest temperature. But these factors separately did not explain any significant portion of data variability. This study do not correspond to Frouz & Finer (2007) conclusions, because there was found no effect of degree of shading in any of seasons, combination of location (A,B,C) with year was also non significant. The only one agreement between these two studies is in effect of year, which was significant in both studies.

In our study the most important factor influencing temperature averages in individual seasons was nest identity. The nest shares all physical characteristics as volume, moisture, altitude and level of shading. Second most important factor was date, which shares insulation level, precipitation and both air and nest temperature. Effect of year on daily average nest temperature was significant in all measured seasons and both years. Older study of Coenen-Strass & col. (1980) reported that temperature in the nest of wood ants depends on climatic conditions i.e. solar radiation, wind, temperature, and humidity of the air and soil, as well as on the ant population density and the size of the hill. These founding corresponds much better with results of present study.

Concerning environmental factors, there was found a significant effect of mean air temperature and nest material moisture in all seasons, whereas volume showed significant effect onto average nest temperature only in summer. Altitude was significant in explaining nest average temperature in summer and autumn. Rain had significant effect onto average inner nest temperature only in spring and summer, effect of nest shading was always non significant. All mean air temperature, nest moisture and volume correlate well with microbial activity. In many previous studies a dependence of microbial heat production of nest material on temperature, moisture, composition of nest material and oxygen concentration was proven (Coenen-Stass & col. 1980, Frouz & col. 1997). Correlation of microbial heat production of nest material and ambient temperature is very strong but it changes with season, the Q10 = 1.8 in winter and 2.5 for summer (Coenen-Strass & col. 1980). During winter, the rate or microbial heat production is fairly constant, but it increases rapidly in spring to four times the winter rate, and remains constant during the summer months (Coenen-Strass & col. 1980).

During the active phase the heat production of nest material from nest center is nearly twice that of peripheral material. Microbial heat production can be partly maintained and regulated by ant activity by loosing and aerating nest interior (Coenen-Strass & col. 1980) and changing nest material chemical properties (Frouz & col. 1997). There is pronounced accumulation of nutrients and easily available substrates and higher pH in ant nests in comparison with surrounding soil, which provides better growing conditions for microorganisms (Dlusskij 1967, Frouz & col. 1997).

Effect of rain was significant only in spring and summer, in this time there is supposed to be enhanced microbial activity in ant nest, which tightly corresponds with moisture (Coenen-Strass & col. 1980). Rain can negatively affect the ant activity, because of limited foraging (Horstman 1987) and thus lower amount of heat brought inside nest by foragers previously exposed to solar radiation (Frouz 2000, 1996). Non significant effect of nest shading may be caused due to loose determination of this category, only three levels were distinguished. Altitude was significant in explaining nest average temperature in summer and autumn. Inner nest temperature of nest in higher altitude seems to be more homogenous than at lower localities, all nests at locality C showed similar characteristics with average difference of mean nest temperature only 1°C among all nests. Nests at localities A and B showed much higher variability in mean nest temperature, the difference could reach 6°C among nests in the same locality.

We could speculate that nests in mountains have to achieve more proper thermal homeostasis because due to harsh mountain conditions, thus selection on more proper thermo-regulation is to occur. But this thought is not supported by data about air temperature because average air temperature did not differ among localities A, B, C. Moreover, temperature fluctuation at the highest locality C was lower than at two other localities. What could vary is the proportion of sunny and cloudy days; in mountains we often observe night and morning foggy cover. According to Raignier (1948) nests of *F. polyctena* after a heavy rain might experience increase in temperature because of a foggy cover after the rain which decreased thermal loss. Another explanation could be often observed inversion weather.

In our study the nest volume was significant only in summer season, although we could expect much stronger dependence. Volume affects inner nest temperature in all seasons through surface-volume ratio (Frouz 1996, 2000, Korb & Linsenmair 1998) and thus the rate of nest heating of cooling. Heat capacity of nest should strongly depend on nest volume too (Frouz 1996). Nest volume is expected to tightly correspond with amount of micro organisms in nest material (Coenen-Strass & col. 1980) and population size (Tschinkel 1987, Coenen-Stras & col. 1980, McIver & col 1997) thus amount of metabolic heat which could be produced from internal nest resources. A possible reason for so low level of nest volume significance should be the fact that we measured only upper part of nest. Previous studies showed that underground part of *Formica* nest could be of same size or even bigger than upper ground mound (Coenen-Strass & col. 1980 Hölldobler & Wilson 1990). We were not able to measure underground nest volume, because digging of nest was not possible neither welcomed in NP Šumava concerning the fatal impact onto ant population survival. Another problem is exact counting of volume, in our study we approximated nest volume with a volume of a cone, which is method used by Frouz (2000). But if nest shape different from normal "moundy" shape, especially in flat nests, the cone approximation may underestimate the real nest volume. In our study this is the case of nests A2 and B3.

To sum up, according to our data we suppose that spring and summer nest thermoregulation relies mostly on internal heat sources, such as microbial heating and heat coming from ant metabolism. Together they result in high and quite stable nest temperatures. This idea is supported by significant effect of nest moisture and volume, as discussed above. The effect of insulation is proposed early in spring, but later it may lose its importance in sense of direct nest heating. However solar radiation may play a role in nest thermoregulation by affecting ant activity and metabolism rate (Eckert 1987, Rosengren & col. 1987, Frouz 2000) and keeping the nest dry (Frouz 2000). When the nest is shaded, nest moisture levels increase and consequently the isolation properties of the nest material become worse. As nest moisture increases, however, microbial heat production, which compensates for the poor isolation properties, also raises (Frouz 2000). However, dry nests are more common than wet nests (Frouz 2000), which should indicate that this is the preferred way of nest thermo-regulation, while nests with high moisture only try to survive unfavorable conditions.

In winter the ants are hibernating in underground chambers (Hölldobler & Wilson 1990) and microorganisms are limited by low temperature (Coenen-Stass & col. 1980), thus we could not count any internal source of nest heat. Nest temperature in winter is only effected by air temperature; nest temperatures are low, but still significantly less fluctuating than ambient temperatures. This is caused by great isolative properties of nest material (Frouz 1996, 2000), which buffer air temperature fluctuations. A nonsignificant effect of volume in winter is hardly to understand and may arise from non appropriate determination of nest volume (see above).

3. Differences of temperature in depth 5, 10, 15cm

Data from spot sampling by hand, which was conducted in period of highest ant activity (April – September), corresponds well with data obtained from whole year datalogger records. Average inner nest temperature in all sampled layers was higher than air temperature, which is in agreement with Frouz (2000).

Temperature in nest of *F.polyctena* rise from 5 to 15cm of nest depth. This idea corresponds perfectly with earlier study of Frouz (2000), who found that the average temperature inside the nest increased from the 5cm layer to 30cm layer. It is also in agreement with Coenen-Strass & col. (1980) whose results show higher heat production of nest material from the center compared with peripheral nest material. This difference is primarily caused by different microbial colonization (different species and population density), due to different nutrients (Frouz & col. 1997). Material from the inner regions contains a higher proportion of friable humus, since microbial activity depends strongly on oxygen, nest interior must be aerated sufficiently. The differences between the heat flows from nest center to surface become smaller during the summer, since the peripheral material is dried out during the warm season (Coenen-Strass & col. 1980).

These results mean that inner nest temperature usually do not directly correspond to level of insulation, as in this case the surface layer should be the hottest one. Temperature difference between 10-5 cm, which is directly proportional to the thermal flow in the nest, is in average positive, the heat flows from inside of nest out. This stress out the importance of inner sources of heat i.e. heat increasing from microbial activity (Coenen- Stass & col. 1980, Frouz & col. 1997) or ant metabolism (Kneitz 1964, Horstmann, 1983, Horstmann & Schmid 1986, Rosengren & col. 1987, Frouz 1996, 2000).

Temperature difference between 10-5cm was similar through whole day, except of midday, where minimal temperature difference was found. We assume that high levels of solar radiation at noon are able to heat the nest material to bigger depth and thus reduce the temperature difference between 10-5cm depth. In 5cm depth there was found bigger temperature fluctuation, which corresponds with Frouz (2000). Nest surface temperature correlates with air temperature, the maximal values of both were reached between 3-6AM. Study of Frouz (2000) concerning nest sampling at different depths do not contain nest surface temperature, thus the comparison is disabled. The temperature difference between 10-5 was also very homogenous in all measured months, except of April, when bigger difference occurred. We could think about effect of big ambient temperature fluctuations together with smaller ant population size, which is not stable yet (Horstmann 1983, 1987, Horstmann & Schmid 1986), and thus not big enough to buffer these fluctuations effectively (Frouz & Finner 2007).

There occurred significant differences of inner nest temperature in all sampled depths (5, 10, 15 cm bellow hill top) among months of measurement (April – September). Consecutive months did not show similar characteristics as expected. September temperatures were totally different from all other months, inner nest temperature was very low, nearly two times lower than summer temperatures. This corresponds with above written founding, that in autumn the nest heating is switched off because it is not needed any more (see discussion, chapter one). May and June were the most different from other months and also form each other, in May the average nest temperature was very low, even lower than in April. This result does not correspond with seasonal average temperature data from datalogger.

However when we look closer to daily nest temperature records, we can observe high nest temperatures at the end of April and beginning of May together with quite high ambient temperatures (often exceeding 15^{o}C) at all sampled locations, followed by approximately two week period of lower nest and air temperature (lower than 10^{o}C). Only few nests did not show a drop of inner nest temperature in this period of cold weather. The May spot sampling was conducted on 20/21st May, which was at the end of cold period, thus the results from this one day do not correspond wit whole month average.

June nest temperatures were the highest of all, which is in agreement with Frouz & Finner (2007). April and June showed similar characteristics concerning temperature in depth 10 and 15cm, but not in 5cm depth. Temperatures in two deeper layers were remarkably high both in April and June. As a possible explanation we could think about increased heat production due to brood incubation and queen egg laying (Steiner 1924, Kneitz 1964, Rosengren & col.1987, Frouz & Finer 2007, Kipyatkov & Schederova 1990, 1985). In wood ants two periods of egg laying occur, first is laid sexual brood in early spring, later in summer worker brood is laid. Eggs laid in spring have more RNA (Bier 1954 in Hölldobler & Wilson 1990), but not all of them necessary hatch as sexuals, because their development also depends on amount of care from nurse workers and quality of nutrition. Early in spring the nutrition relies mostly on fatty reserves from previous summer. For successful development of sexuals a combination of blastogenic and thophogenic factors is needed (Hölldobler & Wilson 1990).

Two consecutive summer months, July and August showed similar temperatures in shallowest nest layer, 5cm bellow nest surface. But no similarities occurred in bigger depths. In the middle of summer the most surface layer temperatures are affected by sun radiation, which is similar for both months. However deep nest temperature did not correspond to sun radiation, it depends more on inner heat sources. Temperature in both 15 and 10 cm depth was steadily decreasing from June to September. This is in agreement with study Frouz & Finer (2007), which showed that early summer nest temperatures were higher than in late summer. Also it suits to our datalogers records data concerning summer temperature slopes

This could be explained by tight correlation between inner nest temperature and ant colony reproduction, in spring or early summer high temperatures are needed for brood development (Kneitz 1964, Coenen-Strass 1985, Rosengren & col. 1987) later in summer there is not so strong need for high temperatures while most of brood is already hatched. Lowering of nest temperature also agrees with founding of Kipyatkov & Schrederova (1985, 1990). The length of queen oviposition period is very stable, some 80days, after this period ant queen enters a diapauses. If egg laying starts in April it will end at the end of June, thus later in summer there is no need for maintenance of high inner nest temperature.

This could also mean that high temperatures in the rest of summer may be only artifact of spring and early summer temperature increase, there need not to be any further ant effort to thermo-regulate. It has been shown that once the inner nest temperature is increased (by ant metabolism or insulation) it could stay high and stable only because of very high microbial heat activity (Coenen-Strass & col. 1986). High air and nest temperatures also speed up ant metabolism (Porter & Tschinkel 1993, Nielsen 1972), which in turn results in increased heat production. A positive feedback works. This could be well possible that the aimed thermoregulation happens only in spring and summer and in late summer it runs due to positive feedback among increased nest temperature, microorganism activity and ant metabolism, lets say thaks to inertia only.

August 2009 and 2010 do not show identical characteristics in no of sampled depth, the biggest difference could be seen between depths of 15cm. Temperature in bigger depth is more independent of air temperature and corresponds with inner heat sources, ie microbial and ant metabolism (Kneitz 1964, Coenenn-Strass & col 1980, Rosengren & col.1987). The contrast in 15cm depth temperature in summer months may be explained by different ant population size of microbial community composition which could be different in two consecutive years More shallow layers (5, 10cm depth) were more similar in between two measured years; we could assume that there occur stronger effect of insulation and air temperature, which was similar in both measured years.

Focusing of whole year pattern of temperature changes results from spot sampling by hand support our earlier conclusions based on data from dataloger records. There was found significant effect of locality and individual nest onto nest average temperature, together with effect of day time and date of study. Nests at locality A express the biggest variability of inner nest temperature among all nest. This variability could be caused by another ambient factor: closeness to water spring. Nests in narrow valley close to water could suffer from water cooling effect; nests placed higher on hill slope are free from this effect. This could be pretty seen on example of nest A1, which was out of valley and exhibit the highest temperatures.

A little surprising was comparison of nests temperature among localities A, B, C. Results showed that nests from locality A are significantly different from B, locality B is different from locality C, but locality A does not differ from locality C (t-test, $p>0.4$). Nests at localities A and C showed significantly ($p<0.05$) higher temperatures in all sampled depths than nest at locality B. Nests from localities A, B and C do not significantly differ in nest volume, insulation or shading level or in seasonal average temperature. Only difference was found in nest material moisture, nests at locality B show lower levels than other two localities, yet this difference is significant between locality B and C ($p=0.04$).

Similarity of nest temperature at the highest and lowest locality should have some explanation in other environmental parameters. It is possible that high temperature of nests is maintained because of disturbing factors, in case of locality C it may be the higher altitude, at locality A it could be cooling effect of close water stream in narrow valley. Both these factors might favor nests with more proper thermoregulation. However, there was found no difference in average air temperature in all localities, air temperature fluctuations at locality C were markedly lower than at two remaining localities (see discussion, chapter 2 and 7).

4. Timing of spring temperature increase

In early spring the inner nest temperatures were low, then a steep temperature increase was observed. In few days nest temperatures rose to 20^{o}C or more, the temperature increase could be more than 10^{o}C a day. The slope of nest temperature increase was much steeper than slope of air temperature changes in spring. Steep spring slope of nest temperature changes could be explained by colony needs in sense of queen oviposition and brood development, which both require high temperatures (Kipyatkov & Schederova 1990, Kneitz 1966, Coenen-Strass 1985, Rosengren & col. 1987, Frouz & Finer 2007). For closer explanation see discussion, chapter 1. In some nests the steep temperature increase was preceded by period of mild temperature increase. The nest temperature increase happened from the end of March to the end of April, the timing of inner nest temperature increase was significantly different both among nests and seasons. This is in agreement with study of Frouz & Finer (2007), who reported that the spring temperature increase in Finland started in all nests in the middle of April, whereas in the Czech Republic temperature started to increase in late February and March in dry nests, and in April in wet nests. Rosengren & col. (1987) recorded steep spring warming of *F.polyctena* nest on 7th April.

Similar pattern was found in study of Frouz & Finer (2007), they reported a massive increase in nest temperature during spring, with average temperature increase up to 1.2°C a day and maximal daily temperature change more than 20°C. The increase of inner nest temperature was significantly faster that the increase in air temperature in all nests (Frouz & Finer (2007). In our study exceptionally quick and high increase of inner nest temperature was found in nest A1, the steep temperature increase was recorded on 26.3.2010 between 11 AM ($t = 9.8$°C) and 5 PM ($t = 18.1$°C), which means an increase of 1.38°C per one hour. In spring 2011 even quicker temperature increase occurred on 29.3.2011 in nest C1, between 11AM and 2PM the temperature jumped form 4.5°C to 26.6°C, making the difference 22.1°C in three hours. Since this day inner nest temperature stayed high and stable.

In agreement with our present study Frouz & Finer (2007) reported variable pattern of spring temperature increase timing in the Czech Republic compared to Finland. They assumed that the increase of nest temperature may be affected by snowmelt. In Finland relatively thick snow cover occurs, which melts rapidly and is followed by consequent start of spring nest heating. Thus in Finland the spring temperature increase started at the same time in all study nests. In the Czech Republic the snow cover is sparer and thinner and, as a consequence, on the top of less shaded nest snow may melt very early. This is supposed to cause difference in timing of spring temperature increase (Frouz & Finer 2007).

Focusing on factors that could explain timing of spring nest temperature increase following model was found: [h.day] = 313.54 - 0.009814*[sun] + 0.01727*[ants] - 5.945*[nest.av] + 101.35*[air.av]. This means that timing of spring nest temperature increase was significantly affected by average spring inner nest temperature, average spring air temperature, nest insulation level and number of ants walking (factors ordered according to amount of explained variability). Last two values were obtained from summer season sampling by hand. This model explained 95.35% of all variability and was significant with $p=0.024$. When deleting average inner nest temperature from the model all other factors lost their significance and there was no factor with significant effect left.

The biggest proportion of variability was explained by average nest temperature in spring. This means that nest starting first in spring nest heating show also higher average inner nest temperature in spring. This is quite logical because they have more days with increased temperature in comparison to nest starting with heating late. High air temperatures connected with sun radiation and insulation could work as trigger which starts autocatalytic process of nest heating (Rosengren & col.1987, Martin 1980).

A cumulative positive feedback of temperature increase was found in ant nests (Coenen-Stass & col. 1980; Rosengren & col. 1987). It was proposed that first little temperature increase caused either by sun radiation, either direct effect of insulation (Kneitz 1966) or heat captured during ant sunning behavior (Zahn 1958); or ant metabolic heat released from fatty reserves of young workers (Martin 1980) cause further increase of microorganism activity and stimulate other workers to wake up and work, which results in massive heat increase in short time period (Rosengren & col.1987).

In agreement with this idea sunning behavior of ants could be observed early in spring (Zahn 1958, Rosengren & col.1987, Frouz 1996, 2000). The higher number of ants sunning the bigger amount of heat brought inside nest. Our results show significant effect of number of foragers, which is proportionally dependent on whole colony size (McIver & col. 1997), onto spring heating. Also that effect of insulation onto spring heating timing was significant, while insulation effect was significant neither concerning number of days with $T>20^{o}C$ nor daily average temperature. Maybe the insulation could be a little different in spring and other seasons, for example because of different sun angle, tree foliation etc.

Effect of altitude was not significant, yet is seems that nests al locality C, the one with highest altitude, show more similar pattern of spring nest temperature increase in both years than nests in two other localities. Neither nest volume nor moisture effect was significant in context of spring heating. This founding is in contrast to majority of theories concerning nest heat production. The spring temperature increase should depend on nest size, while it influences both surface-volume ratio and heat storage capacity, thus the speed of nest cooling and heating (Frouz 1996, 2000); and ant population size (Kneitz 1964, Tschinkel 1987, Horstmann 1983, McIver & col 1997) and amount of microorganisms in nest material (Coenen-Strass & col. 1980, Frouz & col.1997) able to produce metabolic heat. However, some authors deny the importance of ant metabolic heat to maintaining thermal homeostasis of nest, placing the main emphasis on the role of ant mound as a solar collector (Seeley & Heinrich 1981) or microbial heating of nest (Coenen-Strass & col. 1980).

Coenen-Strass & col. (1980) measured thermal productivity of nest material, workers and pupae of wood ants *F.polyctena* and assumed that the heat produced by ant metabolism does not play an important role. His results proclaim the microbial activity of nest material as the main source of heat production. According to Coenen-Strass & col. (1980) there is notable seasonal variation of the heat output of nest material even when measured at stable temperature $30^{o}C$. During winter the rate of microbial heat production is fairly constant, but it increases rapidly in spring to four times the winter rate and remains constant during the summer months. This founding corresponds well with whole year pattern of inner nest temperature changes described above (discussion, chapter 1).

However, Coennen-Strasss & col. (1980) neglect the importance of ant metabolic heat in establishment of nest heat balance. He proclaimed, that during summer the metabolic heat increasing as a by product of ant metabolism would contribute no more than 1% of the total heat output of nest. According to his counts in spring the nest material with a uniform temperature of 10^{o}C and a material humidity of 50% possesses a heat capacity of 3.1 J/ g/C' and produces a heat flow of 0.04 mW/g. That means that in one day (21.5 h) the temperature could increase by 1^{o}C (Coenen-Strass & col. 1980). This is in sharp contrast both with our data showing much more rapid increase of inner nest temperature, even more than 10^{o}C a day and result of Frouz & Finer (2007).

Moreover, Coennen-Strass & col. (1980) added that in midsummer, when the heat balance is established, the heat capacity of nest material amounts to 3.1 J g-1 ^{0}C-' and the heat output is 0.45 mW/g at 24^{0}C. Then it would take only 1.9 h, to raise the temperature from 24 to 25^{0}C. But neither we, nor any of older authors, observed any quick increase in summer nest temperature, which according to Coenen-Strass & col. (1980) might be as high as 12^{o}C a day.

Timing of spring temperature is also influenced by nest moisture (Frouz & Finer 2007), in the Czech Republic the temperature of dry nests started to increase earlier than temperature in wet nests. Our study does not agree with this founding, as there was found no significant correlation of inner nest temperature with nest moisture. Nests with earliest spring increase of inner nest temperature in both years were nest A1 (moisture <20%) and A2 with C1 (moisture >50%). In nests A1 and C1 the spring temperature increase occurred at the end of March, which is approximately one month earlier than in other nests, where temperature increase occurred in last week of April; in nest A2 it was two weeks prior other nests. Nests A1, A2 and C1 do not share any physical characteristics, they differ in both volume and shape; also altitude and level of nest shading are different. Nests A2 and C1 are on sunny place with minimum of shade, but nest A1 is situated in forest. Only similarity is that ants in all these nests are very vital, there was recorded an increase of nest volume in case of A1 and C1 during two years long measuring period.

May be there should be some coincidence with genetic of population. In bees there is genetically given sensibility to temperature, more genetic variable population can reach better thermoregulation (Jones & col. 2004). Also in ants there is variability in temperature sensibility among the workers, some leave the nest very early in spring, possibly seeking the info about day length, than they wake up other workers. The ant hill wake up continuously, in

May some of workers can be still sleeping while majority is already working (Frouz pers.com). The genetic variability of colony should correlate with number of queens in population and number of fertilization (Hölldobler & Wilson 1990). But we do not have any data about genetic variability of measured nests.

Non significance of nest moisture or volume in our study could also coincidence with little amount of available data for comparing timing of spring temperature increase between both years. There are missing data in 2010 because of datalogger loss and in 2011 because too early extraction of dataloggers from nest. Study of Frouz & Finer (2007) reported that the spring temperature increase in Finland started in all nests in the middle of April, whereas in the Czech Republic temperature started to increase in late February and March in dry nests, and in April in wet nests. Thus we hoped when extracting dataloggers at the end of April we will obtain sufficient data about inner nest temperature increase. The opposite was true, because nests in Šumava mountains were late in spring temperature increase compared to nests from Czech lowland near Tábor. The datalogger extraction in 2011 happened on 22nd April, which was at time of ants' biggest building activity. This time was chosen to allow ants to repair nest damage caused by current dataloger extraction and digging of old ones in shortest possible time. This way we hope not to ruin the reproductive effort of colony.

5. Number of days with T>20oC, number of freezing days

According to this study ants maintain markedly high temperature (T>20oC) in their nests for relatively short period of year, approximately 100 days. But this number differed among individual nests, ranging from 62 to 172 days. However no significant difference was found when comparing localities A, B, C, and between two measured years 2009 and 2010. This is in agreement with Frouz & Finer (2007), where the average number of days with increased temperature was 65 – 129. Their results also show no significant difference in number of day T>20°C between locations and seasons, but significant differences occurred among nests. In our study we can observe higher period of increased inner nest temperature (172 day) but this is true only for nest A1. Without this nest the duration of period with T>20°C will drop to maximal value 132 days; this results match more precise with study of Frouz & Finer (2007). Nest with the smallest number of days with T>20°C in whole year 2010 was nest B2, the smallest one, which could mean little microorganisms and smaller ant population size, both these factors could result in low amount of internal heat production. In average day with T>20^{o}C occurred in 80% of all summer days. Only in nest A1 all summer days exhibit higher inner nest temperature than 20^{o}C.

Stable number of days with markedly increased nest temperature found between the Czech Republic and Finland (Frouz & Finer 2007) and also between two localities of different altitude – lowland near Tábor (Frouz 2000, Frouz and Finer 2007) and Šumava mountains (present study) should arise from synchronization of higher nest temperatures with the queen oviposition (Kipyatkov & Schederova 1985, 1990). Kipyatkov & Schederova (1990) gave the duration of endogenously driven egg laying period as 68 – 106 days, which closely corresponds with the duration of period for which ants maintained daily average temperature higher than 20^{o}C.

Focusing of factors influencing number of days with T>20^{o}C significant effect of altitude and average whole year nest temperature was found. This model explained 98.62% of all variability. Neither nest volume nor moisture was significant, but they played an important role in the model, and they increased the proportion of explained variability 99.66%. Shading of nest and summer sun insulation level did not show important effect. Study of Frouz & Finer (2007) did not count any statistical model explaining number of days with temperature increased above 20^{o}C, thus there are no results to compare.

Number of freezing days in whole year 2010 was in average 62.75 days. Again there occurred differences among nests individual nests, with duration of freezing period between 49-83 days. There was also big differences in number of days wit T<0^{o}C between winter 2009/10 and 10/11. Minimal number of freezing days appeared in nest C3, while maximal was found in nest C2. An interesting fact is that both these nests are situated at the same locality, the highest one. There are marked differences in physical characteristics of these nests; volume of nest C3 is the biggest found (1.5m^3) while volume of nest C2 is only 0.22m^3. Nest C3 also shows higher moisture (58.15%) than C2 (35.56%). Moreover, nest C2 is situated in meadow, under single big tree while C3 is in continuous forest. Very exceptional was also nest A2, which got never frozen in winter 2010/11 and spring 2011. Unfortunately we do not have data from whole year 2010 thanks to datalogger loss, thus we cannot compare nest A2 to other nests. But we can assume that it would be the one with lowest number of days wit T<0^{o}C of all. Nest A2 it is very flat, its volume was counted to 0.63m^3, nest moisture is high, reaching 51.58%. We guess that population in this nest is big and very viable according to personal experience from manual measurements.

Model explaining number of days wit T<0^{o}C found as significant only effect of nest volume. Altitude effect was behind the limit of significance ($p = 0.07$), but it also contribute to model explanatory strength; nest volume together with altitude explained 74.57% of variability in number of days with T<0^{o}C. Effect of nest moisture, level of shading and air temperature was not significant. We could assume that freezing of nest is passive process, depending only on volume of nest, let say on surface volume ratio, heat storage capacity and insulative properties of nest material, which are comparable to properties of commercial insolents (Frouz 1996, 2000). Nests with bigger volume are assumed to buffer air temperature fluctuations more effectively. In winter ants are retreated into underground hibernating chambers thus they cannot affect the temperature regime of nest (Rosengren & col. 1987, Hölldobler & Wilson 1990). Moisture effect was not significant in our study, in summer it affects microbial activity significantly, but in winter the microbial activity is limited by low temperatures (Coenen-Strass & col. 1980).

6. Daily temperature regime

Daily temperature regime in summer was quite universal for all nests independent of size or location. The highest inner nest temperature occurred in the evening or during the night, than temperature was decreasing to morning minimum. In some nests the minimum was shifted to midday or early afternoon. Similar results were reported by Frouz & Finer 2007 in dry nests, where the nest temperature peaked in the evening around 6 PM and then decreased during the night. In wet and shaded nests temperature increase was delayed, with maximal temperatures around midnight. This was true for nests both in Finland and the Czech Republic (Frouz & Finer 2007). Concerning these founding, all nests in Šumava Mountains behave as dry, even though in some nests the moisture of nest material could be higher than 50%. Yet no of nests was totally shaded during the whole day.

Evening peak of inner nest temperature corresponds with massive return of foragers (Rosengren & col. 1987, Frouz 2000). The thermal energy accumulated in the workers' bodies together with low thermal capacity of dry nest material (Frouz 1996) could raise the temperature of nest substantially. Similarly, a massive exodus of workers in the morning could result in a temperature depression. A similar effect was observed by Horstmann (1987) after *F. polyctena* workers left the nest, because of the introduction of *Lasius fuliginosus* into the nest. The strong effect of ants leaving a dry nest can be explained by the fact that ants in dry nests are not only an important source of thermal energy (due to metabolic heat production), but also an important heat reservoir (due to high thermal capacity) (Frouz 2000).

Thus the daily regime of inner nest temperature is strongly affected by solar radiation, thanks to ant activity, but not by direct nest heating. As reported in present study 3 the heat in nest flows from inside out (for more details see discussion, chapter 3). The number of foragers going out of nest corresponds tightly with nest surface temperature, which is affected by solar radiation income (see chapter 9). Good foraging conditions thanks to sunny weather can also contribute to better nest homeostasis by higher metabolic rate of well fed ants. Food supply was reported to affect the ability of the nest to produce additional metabolic heat (Coenen-Stass & col.1980, Horstmann 1983, Frouz, 2000).

In early summer pattern with minimal temperature in the morning was more often, later minimal daily temperature was shifted to midday hours. But it did not happen in all nests. A possible explanation is that early in summer there is bigger role of sun radiation, later in summer the importance of metabolic heat produced by ants increase due to population growth (Horstmann 1983, 1987, Horstmann & Schmid 1986). Around midday the biggest ant activity (number of forages on trail) was recorded, thus the number of ants actually staying inside nests is lowered around midday, which may cause decrease of inner nest temperatures despite high ambient temperature. Ants are not important only as a source of metabolic heat, but also as a storage capacity (Frouz 2000).

In autumn there was found no universal temperature regime, the pattern of daily temperature changes could differ between two consecutive days, it could even be inverse. In winter no fluctuations of inner nest temperature occurred. In agreement to our study Frouz & Finer (2007) reported that during winter nest temperatures showed almost no diurnal fluctuation.

Rain could affect the daily temperature regime remarkably; rain could have two opposite effects onto inner nest temperature. In first case due to heavy rain and cold temperatures ants may be hold in the nest (Horstmann 1987); thanks to increased ant aggregation the temperature inside nest is supposed to raise because of increased metabolic production same as increase heat storage capacity (Frouz 2000). In our study we observed this pattern in some nests in late spring or summer; the nest temperature pattern was inverse to air temperature pattern. When air temperatures started to increase again above threshold for ants foraging a massive left on nest occurred, this resulted into a drop of inner nest temperature. Raignier (1948) described an increase of temperature in some nests of *F. polyctena* after a heavy rain. He postulated that the increase in temperature was caused by a foggy cover after the rain which decreased thermal loss.

However after longer period of rain ants could suffer from limited food income because of limited foraging and their metabolism rate decrease (Coenen-Stass & col., 1980, Horstmann 1983, Frouz 2000). Another negative effect of rain is limited amount of solar energy which could heat the nest surface or could be absorbed by ant bodies during foraging. The import of heat energy by ants previously sunbathing is by some authors considered as a major source of heat for dry nest during summer (Frouz 2000), thus when ambient conditions are not suitable for this transport of sun energy into the nest, the nest temperature may drop.

Results from spot sampling conform above mentioned pattern of daily temperature changes. Maximal temperatures occurred in evening or during night, since than a decrease of inner nest temperature was recorded. Minimal values of inner nest temperature occurred either in morning hours or around midday. However most nests from locality B shower slightly different regime of daily temperature changes, with increasing trend from midday minimum to morning maximum. There was observed no drop of inner nest temperature between midnight and morning. In nest B2, the smallest one, the inner nest temperature follows air temperature changes tightly.

Continuous 10 hour measurement revealed differences in daily temperature regime among individual nests. Temperatures in nest A2 were very stable. This nest is flat, so little heat loss could be expected thanks to favorable surface-volume ratio. Volume of nest A2 is $0.63m^{3,}$ but thanks to its shape we could expect some underestimation of our counts; there is also supposed to be big part of nest underground. Nest moisture is high reaching 51.58%, thus the amount of heat produced by microorganisms should be very important in maintaining nest temperature homeostasis (Coenen-Strass & col.1980). We guess that population in this nest is big and very viable according to personal experience from manual measurements.

It could be objected that the daily regime of nest is caused not by ant metabolism but directly by solar radiation income. Due to a big mass of ant mound, its excellent isolative properties (Frouz 1996) and big heat storage capacity, it may take some time to the solar radiation to heat the nest deep enough to nest interior. Similarly this mechanism could be responsible for prolonged cooling of nest with low morning temperatures. But this idea is rebutted by the fact that the heat in nest flows from inside out (see discussion, chapter 3). Only with few exceptions the temperature in deeper layer was higher than temperature of shallower layer at any day time. Also the heat core temperatures exceeded temperature of sampled layers in most cases.

7. Daily fluctuation of inner nest temperature

Fluctuation of nest temperature was significantly lower than air temperature fluctuation in all season and both years. All nests through whole year showed very similar level of inner nest temperature fluctuation. In winter the fluctuation were identical for all nests. Only in summer there could be observed bigger differences among nests in temperature fluctuation (table 3, appendix). Frouz (2000) reported that early in the spring, nest temperature decrease closely follows the decrease in air temperature, because the thermo stability of nest is not well established yet (Kneit 1964). Solar energy is important as the first source of energy able to heat the ant nest (Rosengren & col. 1987, Frouz 2000). Solar energy is trapped both by the nest and the bodies during foraging and then brought inside nest (Zahn 1958). Later during spring, the nest temperature becomes gradually more and more independent of air temperature. The number of ants increases during the season, increases the heat capacity of the nest and makes the metabolic heat production by ants more important (Horstmann 1983, 1987; Horstmann & Schmid 1986, Rosengren & col. 1987).

According to these results we could expect higher fluctuation of inner nest temperature in spring and lower fluctuation in late spring and summer. But we found opposite pattern, with highest nest temperature fluctuation in summer. Summer fluctuation was also the most variable among nests. But also Frouz & Finer (2007) reported significant differences in average daily temperature fluctuation during summer months between individual location and years. Our result show that small nests showed in general bigger fluctuations. This could result from lowered isolation and heat storage capacity of nest with small volume, same as from smaller amount of microorganism (Coenen-Strass & col. 1980, Frouz 2000) and ants able to produce metabolic heat (Horstmann 1983, 1987; Horstmann & Schmid 1986).

In winter we recorded extremely similar levels of nest temperature fluctuation, which is in agreement with previous studies (Rosengren & col. 1987, Frouz & Finer 2007). This pattern could not arise from ant or microbial activity, while both are inhibited by low temperatures. In winter we can count only on effect of snow and nest material isolative properties, which help to buffer big air temperature fluctuations. The high level of inner nest temperature stability, similar for all nests in all seasons and both years, point to strong effect of internal heat sources in nest thermoregulation rather than effect of environmental factors, such as air temperature, precipitation or insulation. From our results it seems that internal heat sources have bigger effect onto nest thermoregulation, because even nests of similar size, placed close to each other, i.e. having similar temperature conditions, often show significant difference in inner nest temperature.

On the contrary, air temperature fluctuation among localities A, B and C were significantly different in all seasons (Tukey HSD test, p<0.001). Air fluctuations at locality C were nearly two times lower than fluctuations of air temperature at localities A and B in spring, autumn and winter. In summer the difference of fluctuation range was smaller.

Founding that at higher altitude there occur smaller air temperature fluctuations was surprising, because it is in contrast both to personal experience and general wisdom that there are more temperature extremes in mountains. Maybe temperature fluctuation on Zelená hora Mountain is buffer by cloud or fog, which occur here very often. Also we could think about a little bit different position of air measuring dataloger. All dataloggers were placed in forest (see metodic), but forest at locality C was continuous whether at localities A and B may be considered sparser. Under continuous canopy there is often higher and more stable temperature than in open plains (Cheng & col. 2008).

Focusing on factors that explain the variability in daily temperature fluctuations in different seasons, the best model i.e. model with highest proportion of explained variability, was counted for summer. In summer the fluctuation was explained by nest identity, average air temperature and air temperature fluctuation, also year and date of study together with altitude and nest moisture showed significant effect. This model explained 98.69% of all variability in nest temperature fluctuation. The only nonsignificant parameters were nest volume and shade category.

Also Frouz & Finer (2007) found significant effect of nest moisture onto daily fluctuation of inner nest temperature. Moisture positively affects activity of microorganisms (Coenen-Strass & col.1980, Frouz & col. 1997, Frouz 2000), thus it helps to keep nest temperature both high and stable. Strange is nonsignificant effect of nest volume onto inner nest temperature fluctuations. We could assume that bigger nests will also have bigger heat storage capacity, more favorable surface-volume ratio and thus lower fluctuations of inner nest temperature. But this is not that case. Nest volume also correlates with size of ant population (Horstmann 1983, 1987) and microbial community (Coenen-Strass & col.1980), but as discussed earlier, our method of volume need not be exact enough because of measuring only upper ground mound and underestimation volume of flat nests.

In autumn there was found significant effect of year of study, followed by nest identity, air temperature fluctuation, average air temperature and altitude. Neither effect of volume nor moisture was significant. In winter and spring similar dependence of nest temperature fluctuation on environmental variables was found. As significant we could count nest identity, average air fluctuation and nest volume; in spring rain effect add to these factors. Nest moisture wasn’t significant in both cases. We could assume that significance of volume in winter and spring corresponds to isolative properties of nest material, which help to puffer big fluctuations

8. Night change of inner nest temperature

The slope of temperature changes during the night (from 24 to 3 hours) was used to compare the individual temperature patterns of inner nest temperature caused by inner heat sources only. This time was in all cases between sun set and sun rise of next day, thus direct effect of insulation was excluded. During that time the ants maintained thermal homeostasis by purely internal sources (Frouz 2000).

The most common pattern of night temperature change in all season was slight decrease in range of -0.4 to 0^{o}C a night. In winter no change of inner nest temperature occurred in more than 50% of all cases. In spring negative slopes count for 55.5% of cases, maximal changes were up to 5^{o}C a night. Significantly higher proportion of negative night temperature change occurred in summer, the proportion of negative slopes was 74.8%. This means that nest temperature decrease was more common than increase, the drop was usually in range 0.1 – 1^{o}C. In autumn negative slopes count again half of all cases, exactly 53.5%. Our results are in agreement with Frouz (2000) who reported that negative slopes, indicating a decrease in temperature during the night, were more frequent than the positive ones. In study comparing thermoregulation of wood ants on south-north gradient (Frouz & Finer 2007), slightly different results occurred. In Finland, the most frequent were situations when night nest temperature slightly decreased or increased, while in the Czech Republic the distribution was bimodal, with rapid decrease being the most common followed by rapid increase (Frouz & Finer 2007).

An explanation of high proportion of negative night temperature change slopes in summer could be as followed: In spring nights the nest interior is heated by ant metabolic heat together with microbial activity, high temperatures are needed for queen egg lying and sexual pupae development (Kneitz 1966, Coenen-Strass 1985, Rosengren & col. 1987, Kipyatkov & Schederova 1990). More ants aggregate inside nest on cold spring nights, when ambient temperature is low, than on summer night, in which ants may stay outside thanks to higher ambient temperature (Frouz 2000). Thus both heat production and heat storage capacity could be lower in summer nights due to lower number of ants present in nest.

Focusing on factors that explain night temperature change, we found significant effect of nest, altitude, year and either air temperature fluctuation or mean air temperature in all seasons. In summer also nest moisture effect was significant. As mentioned above the moisture level correlates strongly with activity of microorganisms (Coenen-Stras & col. 1980, Frouz 2000) which are important source of inner heat. In spring there added significant effect of volume and rain, in autumn of rain only. The volume influence the thermal stability of nest, the bigger volume the bigger heat storage capacity and the lower surface-volume ratio. Both of theses slow down nest cooling (Frouz 2000). Volume also corresponds with population size (Horstman 1983, Tschinkel 1987) and number of ants in turn affects metabolic heat production (Kneizt 1964, Coenen-Strass & col. 1980, Rosengren & col.1987). Rain can affect moisture of nest which could both increase microbial activity and decrease inner nest temperature via decrease of isolative properties of nest material and thus bigger heat loss (Frouz 2000, 1996). Neither volume nor moist were significant in explaining night temperature change in autumn and winter. Our models explained about one quarter of all variability in night temperature change in all seasons.

According to Frouz (2000) the slopes of night temperature changes correlated positively with nest moisture, nest volume and degree of shading, and negatively with the average temperature of individual nests. No correlation was found with the meteorological conditions of individual night. Frouz (2000) postulates that slope of temperature changes during the night seems to be driven by internal nest factors, particularly nest moisture level and nest size. Same results were found in later study investigating diurnal and seasonal fluctuation of wood ant nest temperature in the Czech Republic and Finland (Frouz & Finer 2007). However, they found no effect of rainfall, which is contrary both to our study and the Raignier (1948) hypothesis that night increase in nest temperature is caused by rain and a consequent shield of foggy cover, which reduce thermal loss via heat radiation.

Our results showed significant effect of altitude onto night change of inner nest temperature, but it was in opposite direction than we expected. The change among nests at the lowest locality A (mean -0.33°C a night) was bigger than in localities B and C (mean -0.18 and -0.2°C a night). This correlates quite well with ambient temperature fluctuations which were nearly two times lower at locality C, the one with highest altitude, (mean 4.2°C a day), than at locality A and B (mean 7.3 and 8.0°C day). Strange is combination of small night nest temperature change and high air fluctuation at locality B.

According to personal experiences from field we expected that the night drop of ambient temperature in higher altitude will be stronger than in lowlands. The idea was that harsh mountain climatic conditions force nests to achieve more proper thermal homeostasis, because even a small mistake should be fatal. Thus there only minor fluctuations of inner nest temperature during the night will occur. But our data showed the very opposite results. The average air temperatures did not differ among localities with different altitude, moreover daily temperature fluctuations of air temperature at the highest locality C were the lowest one.

Looking for another possible explanation a word comes to fog and rain. The proportion of sunny and cloudy days could vary among localities with different altitude. In mountains we often observe night and morning foggy cover. According to Raignier (1948) nests of *F. polyctena* after a heavy rain might experience increase in temperature because of a foggy cover after the rain, which decreased thermal loss. Maybe this is the reason for lower decrease in inner nest temperature among nests in high altitude. The year average precipitation (mm) is slightly higher at locality C, but the difference among studied localities is not significant. Unfortunately there are no data about proportion of cloudy or foggy days for testing this hypothesis.

9. Ant activity

Ant activity, measured as a number of foragers walking on trail and number of nest openings, is significantly influenced by surface temperature, altitude, insulation and nest material moisture. Surface temperature, which in turn corresponds with insulation level and air temperature, affects ant activity positively. This is in agreement with Challet & col. (2005), who reported quicker and thus longer distance movement in ant *Messor sancta* at higher temperature. Similar results were found in study of Horstmann (1987), which showed that the number of ants running out of or into the nest depends mainly on temperature (with a maximum near 23°C), and to a small degree on day-time. Disturbing factors are darkness and rainfall. As already mentioned higher temperature in general speeds up the metabolism rate (Nielsen 1972, Coenen-Strass & col 1980, Porter 1988). Concerning our study a simply conclusion can be made: the higher the temperature the more ants walking. Number of foragers going out of nest shows perfect match with axis explained by nest surface temperature.

Ambient temperature also influences number of nest openings. At higher temperature opening more nest entrances may help in reducing the inner nest temperature by increasing air circulation. This principle was described in more ant species, i.e. *F.polyctena* (Horstmann & Schmid 1986) *Acromyrmex heyeri* (Bollazzi & Roces 2010b), Atta sexdens (Roces & Kleineidam 2000). On the other hand, higher temperature means quicker desiccation of nest material (Bollazzi & Roces 2010b), which is not favorable due to negative effect onto larvae development, which have thin water permeable cuticulle. Low moisture is also limiting factor for microorganism activity (Coenen-Strass & col 1980, Frouz 1996).

In our study the results show that opening of nest tunnels corresponds with insulation negatively, the higher the surface temperature the fewer nest entrances are opened. The number of entrances corresponds positively with nest moisture. We can assume that higher surface temperature causes the ants to close nest openings to avoid nest desiccation; ants trade of nest moisture to nest temperature. Bollazzi & Roces (2010b, c) found the very same behavior in thatch ant *A.heyeri*. For each of the three temperatures investigated, the number of openings was significantly lower at low humidity conditions than at high humidity. The fact that workers closed nest openings during the desiccation, despite high temperature, indicates they trade off a thermoregulatory response, i.e. the opening of apertures on the thatch at high temperatures, for maintenance of internal nest humidity (Bollazzi & Roces 2010b,c).

There is a positive feedback between increased ant activity inner nest temperature. During foraging ants are exposed to solar radiation, and in this way, heat energy is imported into the nest by ant bodies (Zahn 1958, Frouz 2000). If environmental conditions are not favorable for foraging, e.g. during rainy days, this source of thermal energy is limited (Rosengren & col. 1987, Frouz & Finner 2007). Also food supply affects ant metabolism and thus the ability of the nest to produce additional metabolic heat (Coenen-Stass & col.1980, Horstmann, 1983, Frouz 2000).

SUMMARY

We investigated thermoregulatory behavior in wood ant genus *Formica*. Average inner nest temperature was higher than ambient temperature in all seasons. Temperature of ant nest was highest in the deepest layer, which means that the heat flows from inside out. The whole year temperature regime was as follows: in spring a steep temperature increase was observed, in summer a plateau of high temperature occurred and during autumn nest temperatures decreased to winter thermal plateau. Pattern of nest temperature was strongly affected by nest identity, date and ambient temperature in all seasons.

Average inner nest temperature in all seasons differed among individual nests, but not between localities with different altitude. Average ambient temperature did not differ between these localities. The most important factors influencing seasonal average nest temperature was nest identity, date, year and nest moisture in all seasons. In summer also nest volume was significant, effect of altitude was significant in summer and autumn. To sum up, during ant activity period the most important effect onto seasonal nest average temperature had those factors, which correspond positively with microbial or ant metabolic heat production.

Timing of spring temperature increase was different among individual nests, it happened from end of March to end of April. A model explaining this difference found significant mean spring nest and air temperature together with insulation level and number of foragers. In spring we can observe sunning behavior of foragers, which enable transport of captured sun radiation to the nest, and thus speed up nest heating. The period of high inner nest temperature ($T>20^{o}C$) was similar in all nests, in average 100 days. This number correlates well with length of queen oviposition period. Factors influencing length of period with increased nest temperature are mean whole year nest temperature and altitude. Neither volume nor moisture effect is significant. This founding is in contrast to previous studies concerning wood ant thermoregulation.

Ant activity counted as number of foragers on trail corresponds positively with nest surface temperature, which in turn is affected by air temperature and insulation. Building activity shows opposite pattern; number of nest openings decrease with high surface temperature, but increase with higher moisture. Ants trade of nest temperature to humidity.

Daily fluctuations of inner nest temperature were lower than air temperature fluctuations. All nest showed very similar level of temperature fluctuation, yet in small nests it was slightly higher. Air temperature fluctuation differed significantly between localities with different altitude, the lowest air temperature fluctuation was found at the highest locality.

Night nest temperature usually slightly decreased overnight. In summer this negative change of nest temperature was more common, probably due to missing heat source and heat storage capacity, while foragers could stay out of nest during summer nights. Both daily temperature fluctuation and night change of inner nest temperature were strongly affected by nest identity, air temperature and year of study. Altitude showed significant effect on night temperature change in all seasons, on daily temperature fluctuation only in summer and autumn. Effect of volume was significant only in spring and winter.

According to our results we could assume that thermal homeostasis of ant nest in spring and summer i.e. in period of ant highest activity is influenced mainly by inner heat sources – microbial activity and ant metabolism. Insulation seems to have direct effect on nest temperature only in early spring; in summer insulation affects nest temperature indirectly, via ant activity. In winter there is pronounced effect of nest volume which corresponds with isolative properties of nest material. Number of freezing days correlates with nest volume only, the bigger the nest the lower the number of freezing days. The thermoregulatory behavior of wood ants is driven by endogenous factors, namely colony needs in sense of queen oviposition and brood development. Both of these require high temperature

CONCLUSION

Main research question of this study was, whether altitude affects thermoregulation behavior of wood ants. Our results showed high stability of thermoregulation behavior, all nests independent of size, moisture or locality showed similar long period of increased nest temperature, approximately 100 days. There is also no difference in average seasonal nest temperature at localities with different altitude. However looking on individual nests separately statistical models revealed significant altitude effect onto length of period with high inner nest temperature. Altitude affects also night change of inner temperature in all seasons and daily fluctuation of nest temperature in summer and autumn. Altitude affects air temperature fluctuation in all seasons too. Thus we can deny our zero hypotheses proclaiming that there is no correlation of thermoregulation behavior with altitude.

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