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Termoregulace mravenců rodu *Formica*, konflikt jedince versus kolonie  
Thermoregulation in ant genus *Formica*, an individual vs. colony conflict

Disertační práce

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**Paper 1** - Kadochová Š., Frouz J. (2014) Red wood ants *Formica polyctena* switch off active thermoregulation of the nest in autumn. *Insectes Sociaux* 61: 297-306

**Paper 2** - Kadochová Š., Frouz J., Tószögyová A (submitted 2017) Seasonal changes in clustering and phototaxis, two important aspects of sun basking behaviour in red wood ants (*Formica polyctena*). *Journal of Insect Behavior*, accepted for major revision

**Paper 3** - Kadochová Š., Frouz J., Roces F. (2017) Sun basking in red wood ants *Formica polyctena* (Hymenoptera, Formicidae): Individual behaviour and temperature-dependent respiration rates. *PLoS ONE* doi:10.1371/journal.pone.0170570

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## Abstract

This thesis deals with thermoregulation in red wood ants, in *Formica rufa* group. Our aim was to better understand the mechanisms by which red wood ants maintain thermal homeostasis in their nests. Red wood ants are known to keep high and stable temperatures in their nests from spring to autumn. Most emphasis is placed on the role of the nest mound as a solar collector or on a heat production by microbial community present in the nest material. However, some researchers believe that wood ants are able of active nest thermoregulation in which they can affect the nest temperature by behavioural reactions, mainly by sun basking, increased metabolic heat production or heat transport.

The thesis consists of three research articles. The first one is focused on the timing of thermoregulation in red wood ants, the second one investigates in more detail one specific aspect of red wood ant thermoregulation - a sun basking behaviour. These two papers provide data from long-term field observations and experiments. The last paper is based on laboratory experiments where we tested a hypothesis resulting from field observations.

Thanks to the field research we found out that ant activity (traffic on ant trails) significantly correlates with nest temperature; once the activity decreased the thermal homeostasis broke down. In agreement with older authors we observed sunning clusters of red wood ants in early spring. We went further and revealed a breaking point at which a positive phototactic behaviour (sun basking) turned to a negative phototaxis (sun avoidance). The breaking point was 42.8 °C which is slightly above the lethal temperature for *F. polyctena*. Our results suggest there is a trade-off between colony and individual needs, i.e. high temperature inside the nest which speeds up brood development and survival of individual workers.

Laboratory observations enabled us to closely follow individual behaviours in sunning clusters. We found out that not all ants take part in sunning and we hypothesised that sun basking might be a specific task. We also measured a metabolic rate (respiration) at the temperatures encountered during sunning. Our results confirm that sunning increases the metabolic rate temporally, but the increase does not persist. Therefore we suggest that a direct heat transport may play a role in the nest thermoregulation of red wood ants.

## **Major questions and hypotheses**

Red wood ant (RWA) genus *Formica* belong to social insect species which have a large effect on the ecosystems they live in via their activities (predation, nest building, honey-dew collection, soil aeration and change of nutrition flow), that's why they are called ecosystem engineers (Dlusskij 1967, Jílková 2015). One of reasons for their success is the ability of nest thermoregulation, which enables quick and safe development for their brood and thus stable supply of working power. Despite the fact that ability of wood ants to keep stable high temperature in their nests was known already from the second half of 20th century (Kneitz 1964, Coenen-Staß et al. 1980, Frouz 2000, Kadochová and Frouz 2013), the mechanism of this phenomenon is still not satisfactory elucidated. In this thesis I want to contribute to better understanding of the phenomena, in particular I want to explore following major question and hypothesis:

### **1. How does a seasonal pattern of the inner nest temperature in wood ant nests look like? Does the temperature have any correlation with the ant activity?**

Previous studies described the pattern of inner nest temperatures mostly in connection to environmental factors (Brandt 1980, Frouz 2000, Stockan and Robinson 2016); however the effect of the ant activity on the nest temperature under field conditions has not been studied yet. Our hypothesis assumes that the ant activity will significantly affect both daily and seasonal pattern of inner nest temperature in RWA nest.

### **2. What is the direction of the heat flow in wood ant nests? Is the nest heated from the inside or outside?**

Several thermoregulatory methods have been proposed for RWA nests. Some authors believe that the nests rely on an outer heat source, the solar radiation (Forel 1920, Brandt 1980, Seeley and Heinrich 1981, Kasimova et al. 2014) and others put emphases on inner heat sources, the ant and microbial respiration (Kneitz 1964, Coenen-Straß et al. 1980, Rosengren et al. 1987, Jílková and Frouz 2014). The direction of the heat flow may reveal which heat source is more important for the nest thermoregulation.

### **3. When do the sunning clusters appear? Does the occurrence of sunning clusters correspond to the increase of inner nest temperature?**

An observation of the sunning behaviour in RWA in spring was mentioned by a few authors (Zahn 1958, Rosengreen et al. 1987, Frouz 2000). Yet no coherent study focused on the timing of sunning cluster occurrence exists. If sunning behaviour is connected with nest thermoregulation, as our hypothesis suppose, it should result into an increase of inner nest temperature. In addition sunning clusters should be formed only in the time when there is a brood present in the nest.

**4. How do the ants behave in the sunning cluster? Are there any differences among individual ant workers?**

Until today we have no report about the individual behaviour of wood ants in the sunning clusters. Our hypothesis supposes that individual ants will form thick clusters to improve their surface-volume ratio and to gain as much heat as possible. After the sunning the ants should move directly to the nest to dissipate the accumulated heat in there as suggested by Zahn (1958). Based on our preliminary observation all ants in colony may take part in sunning behaviour.

**5. Does the body temperature of ants increase during sunning? Does the metabolic rate increase afterwards?**

The heat transport theory (Zahn 1958) assumes that during the sunning the body temperature of ants increases. We tested this hypothesis both in the field and in a laboratory experiment to find out how much the RWA workers can heat themselves. According to Martin (1980) we suppose that sunning behaviour may work as a trigger to lipid catabolism, and thus the metabolic rate of workers should permanently increase after they took part in the sunning.

**6. What are the advantages and disadvantages of the sunning behaviour? Might be the sunning dangerous for individuals?**

Sunning behaviour has been hypothesized to have a thermoregulatory function (Zahn 1958, Rosengren et al. 1987, Frouz 2000). We believe that sunning behaviour is the key for the quick temperature increase observed in RWA nests in early spring which is crucial for the successful brood development. However repeated sunning might be dangerous for the workers because of the high temperatures experienced on the nest surface. Thus there might be a conflict between colony and individual needs.

# General introduction to wood ant thermoregulation

This text introduces to a reader the phenomena of wood ant thermoregulation and highlights how my research fits to overall picture.

## 1. Basics of red wood ant ecology

Red wood ants (RWA) from genus *Formica* inhabit boreal forests in the Palearctic temperate zone (Rosengren and Pamilo 1983, Hölldobler and Wilson 1990). They are famous for building huge nest mounds, often more than 1 meter high (Dlusskij 1967, Frouz and Jílková 2008) which persist for many years of even decades (Hölldobler and Wilson 1990). RWA are polydomous and polygynous, which means they form huge colonies with tens or hundreds nests and there can be more than one queen in each nest (Rosengren and Pamilo 1983, Ellis and Robinson 2014, Stockan and Robinson 2016, **Paper 2** - our field colony had 35 active nests). This makes red wood ant nests virtually immortal, since there is a multiple generation overlap and the mother queens are continuously replaced.

RWA are omnivores; they prey for insect, small invertebrates and for aphid honeydew (Horstman 1974, Domish et al. 2009, Jílková and Frouz 2014). The amount of prey brought inside the nest is huge, annually approximated to 25 kg of dry mass of prey (Frouz et al. 1997) and 13–16 kg of honeydew (Jílková et al. 2012). Wood ants accumulate in their mounds large amounts of organic matter, rich in carbon and nitrogen; it's decomposition leads to sustained soil enrichment which affect nutrient cycles and tree growth as well (Horstmann 1974, Frouz et al. 2008, Kilpeläinen et al. 2009). That is why RWA are often called ecosystem engineers (Dlusskij 1967, Sorvari 2013, Jílková 2015, Stockan and Robinson 2016).

## 2. Principles and importance of thermoregulation

### 2.1 Thermoregulation in social insect

Nests of social insects serve not only as a shelter for adults but also as an incubator for the brood (Wilson 1971, Jones and Oldroyd 2006). Inner nest temperature is a vital factor, since it affects the rate of brood development (Porter 1988), the rate of movement and foraging (Porter and Tschinkel 1987, Challet et al. 2005), and the rate of food and oxygen consumption (Coenen-Stass 1980, **Paper 3**). Thus higher temperatures can be advantageous for the colony fitness thanks to a quicker brood development and disadvantageous at the same time because of higher energy expenditure (Brian 1973, Hölldobler and Wilson 1990).

Several ways of nest thermoregulation are applied among social insect species. First it is necessary to build some kind of a nest. According to Jones and Oldroyd (2006) insect can build the nest in a cavity and rely on its protection abilities, migrate the nest frequently according to ambient conditions or build a stable nest and migrate the brood within the nest along a thermal gradient. Second part of the thermoregulation are behavioural reactions of workers; they ensure brood translocation, heating when



the nest is cold or ventilation when the nest becomes too hot (Seeley and Heinrich 1981, Jones and Oldroyd 2006).

Most well known example of nest thermoregulation are the hives of *Apis mellifera*. Honeybee workers are able to produce metabolic heat by vibration of wing muscles (Lindauer 1954, Wilson 1971, Heinrich 1993, Seeley 2009). This way they can increase the temperature in the whole nest or even in individually selected brood combs, by pressing the thorax to a brood cap or a wall (Kleinhenz et al. 2003). Honey bees also apply thermoregulation in their temporal swarms, keeping the bees inside the swarm core warm and ready to fly (Heinrich 1981). They are very effective in nest cooling too, they ventilate nest with their wings, spread water droplets to support evaporative cooling and shield the brood combs with their own bodies if needed (Seeley and Heinrich 1981). Other species of winged social insect apply similar techniques as honeybees (Jones and Oldroyd 2006).

## **2.2 Thermoregulation in ants**

Ants lack wings, except of the short swarming period, so they had to evolve other ways how to achieve high temperatures in their nests. In moderate climates most ants build underground nests benefitting from lower temperature fluctuation in the soil. In addition some ant species nest under rocks or stones which serve as heat collectors or they use a layer of leaf litter as an isolative buffer (Hölldobler and Wilson 1990). Better regulation of inner nest temperature is achieved in the mound-building species of the genera *Atta*, *Acromyrmex*, *Myrmicaria*, *Pogonomyrmex*, *Solenopsis*, *Iridomyrmex*, *Formica* and *Lasius* (Kadochová and Frouz 2013).

Nest mounds usually have large spatiotemporal variations in temperature, being heated from one side by solar radiation. This way a temperature gradient is created and worker ants select the optimal temperature for the brood development and perform a brood displacement (Porter 1988, Cassil et al. 2002, Kadochová and Frouz 2013). Examples of such nests are soil mounds of *Solenopsis invicta* (Penick and Tschinkel 2008), *Camponotus mus* (Roces and Núñez 1989) or nests of *Acromyrmex heyeri* (Bollazzi and Roces 2002) which build upper-ground hills from soil and dead vegetation, i.e. materials with higher isolative properties, similarly to *Formica polyctena*.

## **2.3. Red wood ants – thermoregulation and life cycle**

The nest thermoregulation in RWA is based on the combination of passive and active thermoregulatory mechanisms. An elaborated nest structure together with behavioural reactions enable wood ants to maintain a precise thermal homeostasis in their nests (Jones and Oldroyd 2006, Kadochová and Frouz 2013, **Paper 1 - 3**).

From spring to autumn stable inner temperatures above 20 °C are maintained in the centre of RWA nests regardless of weather conditions (Kneitz 1964, Rosengren et al. 1987, Frouz 2000, Stockan and Robinson 2016, **Paper 1, 2**). The place with stable temperatures is called a heat core and it is where the brood is placed. Outer parts of the nest are a subject to bigger temperature fluctuations. High

temperatures are usually maintained for a limited time period, going from 65 - 129 days (Frouz and Finer 2017), in our study up to 172 days in one nest (**Paper 1**). This pattern is similar over latitudinal and altitudinal gradient (Frouz and Finer 2007, Kadochová 2011, **Paper 1, 2**).

It is believed that the nest thermoregulation in RWA nests closely correspond to a reproductive cycle of queen who undergoes regular shifts between reproduction and diapauses (Kipyatkov and Schederova 1990, Cherix et al. 2006). Those cycles are very stable; they persist even under stable laboratory conditions (constant temperature and photoperiod). It was postulated that ants maintain high temperatures only during the queen's reproduction phase, which last approximately 100 days (Kipyatkov and Schederova 1990). Once the queen enters the diapauses the inner nest temperature drops regardless of ambient temperature. Our research has shown that at the end of September RWA switch from active to passive thermoregulation (**Paper 1**). In September the ants still perform foraging and other activities, but they resign from maintaining the thermal homeostasis of the nest. The inner nest temperature can drop even more quickly than the air temperature (**Paper 1**).

### **3. Mechanisms applied in RWA nests thermoregulation**

#### **3.1. Nest structure as a solar collector**

Most important for RWA thermoregulation is the construction of upper ground nest mound. It is built from a mixture of soil, coniferous needles, small twigs, pebbles and pieces of resin (Hölldobler and Wilson 1990, Castella et al. 2008). This material shows unique thermal properties such as a big heat storage capacity and an excellent isolative ability (Coenen-Stass et al. 1980, Frouz 2000).

According to many authors (Forel 1920, Brandt 1980, Seeley and Heinrich 1981, Rosengren et al. 1987) the nest mound serves as a solar collector. Wood ant nests, despite their name, can be seldom found in dense high stand forest; most of the nests are placed at forest edges, clear cuts or other openings (Dlusskij 1967, Hölldobler and Wilson 1990, Kilpeläinen et al. 2008), where there is enough solar radiation available (**Paper 2**). Young queens of *Formica* ants preferably chose well insulated places for new nest establishment (Punttila 1996, Chen and Robinson 2015). If young nests are overgrown by a dense forest they rarely persist (Sorvari 2013). However the nesting preferences may differ according to the individual specie (Stockan and Robinson 2016).

The shape of the nests mound is adapted to harvest of solar radiation, the south slope is usually longer and the slope more gentle (Forel 1920). For this reason ant nests were used as a primitive compass by mountaineers (Hölldobler and Wilson 1990). Newt studies and mathematical models confirm that the shape of RWA nest mound is ideal for solar energy trapping (Kasimova et al. 2014). Compared to underground nests, mounds absorb heat more quickly both in the direct sun and in the shade (Penick and Tschinkel 2008). Our field measurements show that solar income often exceeds 10 000 LUX in summer and temperatures of the insulated nest surface can reach more than 60 °C (**Paper 2**).

The solar income was shown to significantly affect the inner nest temperature (Rosengren et al. 1987, Frouz 2000, Jílková et al. 2015a, **Paper 1**) yet our results suggest that the solar radiation is not the

main source of heat for mature RWA nests (**Paper 1**). Thermal flow usually goes from inside out, highlighting the importance of inner heat sources. Reverse heat flow, with heat entering the nest from outside, was observed only at midday on days with high solar income (**Paper 1**). The reason for this discrepancy with the original solar collector theory could be the excellent isolative properties of nest material. In dry nests there is a low thermal conductivity, the heat from the nest surface penetrate slowly do deep levels (Frouz 1996). This could be an advantage in summer months, when the solar income is too high and it could cause overheating of the brood. On the other hand in spring this could be a problem, since the solar heat is needed.

We already know that thermal conductivity depends on a level of moisture (Coenen-Staß 1980, Frouz 1996, 2000). In spring the nests are wet after the snowmelt and a long period of winter inactivity which increases the thermal conductivity of nest material. Thus in spring the heat coming from solar radiation can penetrate to deeper nest layers more easily. During the year with increasing solar income and ant activities (nest reconstruction, tunnel building and aeration of nest material) nests become drier, the isolative capacity increases and the thermal conductivity lowers (Frouz 2000, Jílková and Frouz 2014).

### **3.2. Nest material and microbial respiration**

Other researches place emphasis on inner heat sources. One of them is the respiration of microbial community present in the nest material (Coenen-Straß et al. 1980, Frouz et al. 1997, Domisch et al. 2008). Respiration of RWA nests is significantly higher than the respiration of the surrounding forest floor (Risch et al. 2005, Jílková et al. 2015b). Inside RWA nests there can live fungi and different types of bacteria (Actinobacteria, Gram-positive and negative bacteria) (Jílková et al. 2015a, Stockan and Robinson 2016).

The thermoregulation via microbial heating was proposed in 1915 by Wasmann. In 1980 the theory was confirmed in a laboratory experiment, showing that natural nest material (with microorganisms) produces measurable amounts of heat whereas a sterilized nest material (without microorganisms) does not (Coenen-Straß et al. 1980). The respiration of nest microorganism and consequent heat production changes during the year, because of changes in the temperature, moisture and nutrition supply. The highest nest respiration occurs in summer (Coenen-Straß et al. 1980, Jílková et al. 2015b). Ants can affect the microbial respiration via their own activities such as nest material aeration, fresh plant material and honeydew supply (Wilson 1971, Jílková et al. 2014) or their own metabolic heat production (Kneitz 1964, Coenen-Straß et al. 1980). Temperature has a significant positive effect on the microbial respiration, which creates a positive feedback loop (Rosengren et al. 1987, Jílková et al. 2015a). The microbial respiration increases also with an increasing moisture content, in wet nests the respiration is three times higher than in dry nests (Frouz 2000, Jílková and Frouz 2014). However wet nests also have a bigger thermal conductivity which may lead to substantial heat losses, especially at

night (Frouz 2000). Increased microbial respiration together with a bigger heat storage capacity is supposed to compensate for the heat losses in wet nests (Coenen-Staß et al. 1980, Horstmann 1990). Yet despite the original suggestion of Coenen-Straß (1980) microbial heating seems not to be the main heat source for RWA nests. New field and laboratory studies show that the microbial respiration represent only 25% compared to the ant respiration (Jílková and Frouz 2014). Still the microbial respiration is important for early thermal homeostasis establishment, since both ants and microbes contribute to the spring increase of inner nest temperature.

### 3.3. Metabolic heat of ants

Another theory claims that the ants themselves are able of a metabolic heat production (Steiner 1924, Kneitz 1964, Rosengren et al. 1987, Jílková and Frouz 2014) despite the fact they do not have flying mussels which are used for the heat production in bees (Lindauer 1954, Johnson and Oldroyd 2006). We monitored the temperature in different depths of RWA nests during whole season and find out that the heat usually flows from inside out (**Paper 1**), which suggests that the nest thermoregulation indeed rely on inner heat sources.

Rosengren et al. (1987) observed an increase of inner nest temperature on cold spring days, with ambient temperature limiting ants from outdoor activities. He postulated that it could result from the metabolic heat produced by ant workers clustered in the nest centre. Similar results were shown by Frouz & Finner (2007). Repeatedly a quick spring increase of inner temperature in RWA nests was described (Zahn 1958, Rosengren et al. 1987, Frouz 2000, Jílková et al. 20015a, **Paper 2**), sometimes even in snow covered nests (Rosengren et al. 1987, Cherix et al. 2006) when the heat cannot be obtained from solar radiation yet.

Martin (1980) suggested that in large nests of *Formica rufa* nests the spring nest heating could start as an autocatalytic process that relies on utilization of the lipid reserves in young workers. He believed that sun basking behaviour serve as a trigger to a catabolic lipid degradation which leads to the increased heat production (Martin 1980). We tested this theory in a laboratory experiment in which we measured a metabolic rate (respiration) of ants before and after the sunning and we found no support for Martin's theory (**Paper 3**). The metabolic rate (respiration) increased with increasing temperature experienced during the sunning but this increase doesn't persist. Just two days after the sunning event the metabolic rate was the same as before the sunning (**Paper 3**).

Recent respirometric measurement show that the ants are responsible for 75% of CO<sub>2</sub> production in RWA nests and thus the metabolic heat produced by workers is responsible for the high inner nest temperatures (Jílková and Frouz 2014). Moreover the ants can adjust their respiration and thus the metabolic heat production to the actual temperature needs of the nest; in cold environments the respiration of both ants and microbes is higher to achieve the target nest temperature (Jílková et al. 2015a). Our field results confirm that the nest thermoregulation in RWA is connected with the ant

activity. The inner nest temperature is significantly affected by the number of ants coming to the nest (**Paper 1**), as was postulated in Frouz 2000.

This means that the nest thermoregulation in RWA is an active process affected by ant presence and activity. The active thermal homeostases is maintained for a limited time period only, in autumn the thermoregulation is switched off despite favourable weather condition (**Paper 1**).

### **3.4. Sun-basking behaviour and heat transport**

There is one more way how the wood ants can actively affect the temperature balance in their nest, a specific sun-basking behaviour which has been described in RWA by a few authors (Zahn 1958, Rosengreen & col. 1987, Frouz 2000, Frouz and Finer 2007). In the spring ant workers have been observed basking in the sun and later entering the nest interior. It is supposed that while sunbasking the ant bodies accumulate sufficient amounts of solar heat which is later transported inside the nest and herein it is dissipated (Zahn 1958, Rosengreen et al. 1987, Frouz 2000). Thick sunning clusters, with ants packed so densely together than no underlying material is visible, can be observed in spring only (**Paper 2**).

As already mentioned the nest material of RWA mounds has excellent isolative properties (Coenen-Staß et al. 1980, Frouz 1996), which slow down the penetration of solar heat, especially in dry nests. The heat transport in ant bodies might be an elegant solution to this problem. Ant bodies are dark, they contain relatively big amount of water, which makes them an ideal mean for the heat transport (Zahn 1958). Sunning in clusters improve the surface volume ratio, making the process of heat trapping even more effective.

We studied the sun-basking behaviour with an infrared camera both in the field (**Paper 2**) and in the laboratory (**Paper 3**) under an artificial heat source. In agreement with older studies we observed thick sunning clusters in spring only, suggesting the sun-basking behaviour is a time limited phenomenon. In summer the sun-basking behaviour disappeared and the ants switched to sun avoidance. At that time nest surface temperatures were often higher than 40 °C which exceeds the lethal limit for *F. polytenua* ( $40.67 \pm 0.35$  °C) (**Paper 2**). We assume that the change from positive to negative phototaxis serves as a safety mechanism avoiding the death of workers.

Individual behaviour of RWA workers in sunning clusters was studied under laboratory conditions. We found out that the ants spent in average 10 minutes in the sunning cluster, during sunbasking their body temperature increased (up to 30 °C depending on the power of the heat source) and after sunbasking they usually returned directly to the nest (**Paper 3**). Pilot studies on the physical heat transport in workers' bodies revealed that a single ant may walk long distances (more than 50 cm) before it loses the accumulated heat (Muth 2012, our unpublished data).

We believe that the sunning behaviour of RWA is an inevitable part of the unique thermoregulatory mechanism of heat transport (Zahn 1958), which helps to establish the thermal homeostasis of RWA nests in early spring. The heat carried into the nest in ant bodies may start the positive feedback loop –

an increased temperature in small part of the nest will lead to an increased metabolic heat production by both ants and microorganisms (Jílková and Frouz 2014). This way the temperature hike observed in spring might be explained.

### **3.5. Other theories**

Dr. Gabriele Berberich, a geologist from university of Duisburg-Essen, believes that position of RWA nests is affected by tectonic situation of a given locality. Her theory postulates that RWA place their nests on geological faults, where there is a leakage of carbon dioxide, radon, helium and other gases (Berberich et al. 2013). Temperature measurements at geological faults showed a slight increase of soil temperature (1 or 2 °C, Berberich pers. com) compared to neighbouring areas which, from the biological point of view, might be very important for the nest site selection and thermoregulation.

Moreover Berberich et al. (2013) claim that RWA can predict earthquakes, since their behaviour routine of ants changes several hours before the earthquake. According to their results both the nocturnal rest phase and daily activity were suppressed by the seismic activity. Yet other researchers (Apostol et al. 2016) failed to confirm this theory. In Romania the RWA do not place nests on geological faults and their behaviour seems unchanged by the coming earthquake.

## **4. Individuals and societies**

### **4.1. Role of individuals in insect societies**

Based on life strategies insect is often divided to solitary and social. In those insect species, which live in solitary, each individual perform the same tasks during its life. The single individual has to forage and feed itself, seek for a shelter, keep safe, reproduce and care for its offspring (Wilson 1971). On the other hand in communities of social insect the roles are differentiated, the reproduction is strictly separated from other tasks such as foraging, nest building or brood rearing (Trivers and Hare 1976, Gordon 1966, Schwander et al. 2005). However there are many transitional stages with less strict separation on the way from primitive cooperation of few female individuals to the true eusocial societies (Oster and Wilson 1987, Žďárek 2013).

An ideal example of eusocial life is a honeybee, *Apis mellifera*, on which most models of social behaviour have been described. In a bee hive there is only one queen, which is the only one in the whole society permitted to reproduce. All other bees are her daughters, which spent whole life working for the good of the society without a chance to reproduce and pass their genes to other generations (Sakagami 1954, Wilson 1971, Bonabeau et al. 1997, Seeley 2009). This unselfish behaviour was explained in 1964 by the Hamilton rule (Hamilton 1964) and later by the Haplo-diploid theory (Trivers and Hare 1976).

The haplo-diploid kin selection assumes that by rearing their own sisters the workers of bees, ants and other haplo-diploid insect can pass more genes (75%) to the next generation than by rearing their own offspring (50%) (Trivers and Hare 1976, Foster et al. 2006). Because the worker is more related to her

sisters (the queen's daughters) than to her own offspring the direct reproduction of workers is disadvantageous. The most effective way to increase it's own evolutionary fitness is helping to rear the queen's offspring (Trivers and Hare 1976). From this point of view the individual life of a worker has no value and it is worth to sacrifice it for the benefit of the colony.

That is why *Formica* workers will evacuate larvae and pupae under the enemy attack better than escape (Tamarri et al. 2009), honeybees will heat themselves to death when enclustering a hornet in an "antipredatory own" (Ono et al. 1995) and hundreds of fire ants will drown when creating floating pontoons during a colony saving at floods (Mlota et al. 2011).

#### **4.2 Task division and thermoregulation**

In many eusocial communities we can observe a task specific division of labour (Wilson 1971, Bonabeau 1997). The labour division can be driven by age, morphologic or genetic differences among workers, same as by their social interactions or previous experience (Robinson 1992, Calderone 1998, Jones et al. 2004, Ravary 2007, Robinson et al. 2009). In most species the labour division is flexible and thus it allows the colony to quickly react to actual colony needs, since one individual can perform several tasks (Robinson et al. 2009).

There are specific tasks related to the nest thermoregulation. In the honeybee the brood tending workers are usually the young ones (Wilson 1971, Page and Peng 2001, Seeley 2009). They monitor the temperature of brood combs very carefully and if it drops below demanded optimum they apply heating strategies (Bujok et al. 2002, Jones and Oldroyd 2006). The workers press their thorax to the cap of an individual brood cell and they increase the thorax temperature by muscle vibration (Esch 1960, Heinrich 1993, Jones et al. 2004). In some cases they enter empty cells adjacent to the brood cell and start to heat up their body, this time heating six neighbouring combs at the same time (Bujok et al. 2002, Kleinhenz et al. 2003).

In case of extreme heat the workers would ventilate the nest by wing fanning, increase evaporative cooling by spreading of water droplets or even shield the combs with their own bodies (Lindauer 1954, Heinrich 1993, Starks et al. 2004). Different thresholds for different thermoregulatory activities assure a balanced homeostasis of the nest; some individuals react to the slightest temperature change while others will respond only at a sever temperature increase (Theraulaz et al. 1998, Jones et al. 2004).

In termites the temperature control and nest building tasks are tightly connected, since the thermal homeostasis in the termite mound is maintained mostly thanks to the special nest architecture (Korb and Linsenmair 1998, Jones and Oldroyd 2006). In a cold environment nests have thick walls and limited amount of turrets which keep the inner temperature high, while in a hot environment the nest walls are thin and there are many turrets enabling both heat and gas exchange. An efficient ventilation system assures gas exchange and temperature homeostasis (Korb and Linsenmair 1998).

In wood ant genus *Formica* the workers perform tasks as foragers, brood tenders, transport specialists, nest builders and nest guardians similarly to other ant species (Wilson 1971, Robinson et al. 2009). Task division based on age was documented in RWA, with young workers performing the inside-nest tasks and older one foraging outside (Rosengren 1971, Otto 2005). However this rule is very flexible, each worker can perform any task when needed (Zakharov 1980, Jones et al. 2004). The task division happens thanks to the different reaction thresholds in individual workers. Ant workers react to stimuli in a given sequence of priorities according to their importance for the colony, this way a flexible reaction to colony needs is ensured (Theraulaz et al. 1998, Robinson et al. 2009).

A special task occurs in RWA - aphid tending, where the ants patrol over the aphids, guard them and if needed move them to safer and more productive areas (Horstmann 1974, Otto 2005, Novgorodova 2005, Stockan and Robinson 2016). We suggest, there might be one more specific task allocation – sunbasking (**Paper 3**). According to our results not all ants from the colony take part in the sunbasking and from those who do, some spent significantly more time sunning. Those might be considered sunning specialists. However we found no physiological or morphological difference between the ants which took part in the sunning and which did not (**Paper 3**). The explanation might be in higher levels of genetic diversity in workers caused by multiple-queen colonies in RWA, which result in less polymorphic workers (Swchwander et al. 2005).

#### 4.3 Selfish behaviour

However well organized the RWA society is, it is far from ideal. The ants from *Formica rufa* group are polygynous and polydomous, there is often more than one queen in one nest and she practices a multiple mating (Rosengren and Pamilo 1983). This means that in one nest the ant workers are a big mixture of genes from different parents which compromise the haplo-diploid kin selection. In RWA nests many workers are not sterile, they often lay unfertilized eggs (Helanterä and Sundström 2007). Those can hatch in males which can mate with other workers and pass the genes of “selfish” worker-mothers further. Workers’ egg laying and selfish behaviour was documented in *Formica fusca* (Hannonen and Sundström 2003), *Leptothorax acervorum* (Hammond et al. 2002), fire ant *Solenopsis invicta* (Keller and Ross 1998), or ponerine ant *Gnamptogenys* (Gobin et al. 1999). It happens also in the “example” of eusocial society, the *Apis mellifera* (Sakagami 1954).

To lay eggs the workers need to survive and thus even more selfish behaviour, preventing workers from death, may be expected in ant societies. Focusing on the nest thermoregulation in RWA the sunning behaviour can be viewed as potentially harmful to ant workers. Temperatures on the sun exposed nest surface often exceed the lethal limit for *F. polyctena* (**Paper 2**), especially in summer months. At temperatures higher than 40 °C protein denaturation and metabolic restriction may happen (Heinrich 1995, Neven 2000). Sun avoidance was documented in RWA in summer on occasions that may compromise the worker survival (**Paper 2**).



Based on our results we suggest there is a trade of between colony and individual needs in RWA connected with early nest thermoregulation, i.e. with sun-basking behaviour. Occurrence of sunning clusters depends on inner nest temperature, nest size and population size. Sunning clusters appear when the inner nest temperature is low, thus reflecting the colony need for thermo-regulation. However the phototactic reaction of workers, the decision to be or not to be on sun exposed nest surface, is affected by the temperature of nest surface, i.e. what the workers can endure (**Paper 2**). There is a potential conflict between the colony need for thermoregulation and the individual survival. Even when nest surface temperatures are below the lethal limit the sun-basking behaviour may reduce the survival of the ant workers. During the sunning the body temperature of workers increases markedly and so does the respiratory rate (Brian 1973, Jilková et al. 2015a, **Paper 3**). This means higher energy expenditure and a shortened life span for those workers which took part in the sunning (Calabi and Porter 1989). If RWA workers want to lay eggs they should be selfish and they should avoid sunning. Our laboratory experiments showed that some ant workers indeed avoid sunning (**Paper 3**). There are two possible explanations for it, either the sunning is a specialized task, or the avoidance is a selfish act.

## Major findings

### **1. How does a seasonal pattern of the inner nest temperature in wood ant nests look like? Does the temperature have any correlation with the ant activity?**

In coherence with other authors (Rosengren et al. 1987, Frouz 2000, Frouz and Finner 2007, Jílková et al. 20015a) we observed that inner nest temperature of RWA nest is maintained high (over 20 °C) and stable from spring to autumn (**Paper 1, Paper 2**). In spring there is a steep increase and in autumn a steep drop of inner nest temperature which is quicker than that of air temperature (**Paper 1**). From April to August the inner nest temperature is significantly affected by ant activity, namely by number of ants going into the nest. This period of active maintenance of the inner temperature is limited to maximally 172 days (**Paper 1**). In September the nest temperature drops regardless of weather conditions, thus we assume the ants cease their thermoregulatory activities and the nest switches to passive thermoregulation.

### **2. What is the direction of the heat flow in wood ant nests? Is the nest heated from the inside or outside?**

We measured the inner nest temperature manually in 3 different depths (5, 10 and 15 cm below the nest apex) and the results show that the heat flow usually goes from inside out (**Paper 1**). A reverse situation was observed only on days with high solar income (usually summer) during midday. Thus our results confirm that RWA nests rely primarily on inner heat sources as suggested by previous authors (Kneitz 1964, Coenen-Straß et al. 1980, Rosengren et al. 1987, Jílková and Frouz 2014).

### **3. When do the sunning clusters appear? Does the occurrence of sunning clusters correspond to the increase of inner nest temperature?**

Our results show that sunning behaviour of RWA is a time-limited phenomenon. The ants create sunning clusters only in spring, when both air and inner nest temperatures are low (**Paper 2**), preferably at days with good weather conditions (high solar income), when a quick heating of the ants in the clusters may happen. The timing of cluster occurrence is affected by both ambient factors and nest parameters. The most important factor is the inner nest temperature; second the nest size and population size, all those factors reflecting the inner needs of ant colony in sense of thermoregulation (**Paper 2**).

### **4. How do the ants behave in the sunning cluster? Are there any differences among individual ant workers?**

We observed behaviour of ants in sunning clusters both in the field (**Paper 2**) and in the laboratory (**Paper 3**). The behaviour of ants in sunning clusters is similar to those of honeybees in a swarm; the cluster is not stable, it is still changing with ants moving from the outside in. Under the laboratory

conditions one sunning event lasted in average 10 minutes; after the sunning the ants usually went back into to nest or they headed for a water source and then they continued in sunning (**Paper 3**). Thanks to an individual marking of workers we found out that not all ants take part in the sunning – there are sunning and non-sunning individuals in a RWA colony (**Paper 3**). Yet morphological and physiological measurements did not reveal any difference between “sunnings” and “non-sunnings”. They differ neither in the body size (body mass, head width) nor in the respiration rate (**Paper 3**).

### **5. Does the body temperature of ants increase during sunning? Does the metabolic rate increase afterwards?**

Our observation with an infrared camera confirmed that during sunbasking the body temperature of ants increases. In the laboratory experiment (temperature controlled box maintained at 10 °C with a 150W IR lamp as a heat source) we observed ants heated up to 30 °C (**Paper 3**) in the field the ant body temperature could reach 40 °C (**Paper 2**). The respiratory rate (production of CO<sub>2</sub>) of ant workers during and shortly after the sunning increased (**Paper 3**); the respiration at 35 °C was ten times higher than at 5 °C. However, when we compared respiration rates of the sunning workers before and two days after the sunning experiment we found no difference. Thus we can deny Martin’s theory (1980) that the sunning behaviour works as a metabolic trigger for catabolic lipid degradation.

### **6. What are the advantages and disadvantages of the sunning behaviour? Might be the sunning dangerous for individuals?**

Sunning behaviour appears only for a limited time period (**Paper 2**), which is consistent with the time of sexual brood presence in RWA nests (Kipyatkov and Schederova 1990, Cherix et al. 2006). This confirms the hypothesis that sunning behavior has a thermoregulatory function. The sunning is advantageous for the colony since it helps to establish high inner nest temperatures needed for sexual brood development (Wilson 1971). However according to our results sunning might compromise workers survival. In a field we recorded nest surface temperatures often exceeding 40 °C, which is beyond the lethal limit for *F.polyctena* (40.67± 0.35 °C.) In the most extreme case the temperature of sun exposed nest surface reached 67.2 °C (**Paper 2**). Certainly at that time the ants were not present on the nest surface, in summer we observed strict sun avoidance, which presumably serve as a self protecting mechanism for ant workers. Still the temperature increase experienced during sunning is costly for workers because of high respiratory rate and energy expenditure (Brian 1973, Jílková et al. 2015a, **Paper 3**) and it may also shorten their life span (Calabi and Porter 1989).

## Conclusion

Our research has shown that the ability to keep high stable temperatures inside wood ant nests is given not only by physical properties of the nest mound or a passive income of solar radiation. The opposite is true. The ants can affect the inner nest temperature via their activity; they can lower the temperature (air circulation, galleries reconstruction, change of isolative layer thickness) or increase the temperature (Jones and Oldroyd 2006, Kadochová and Frouz 2013). The nest thermoregulation relies on inner heat sources, the heat flows from inside out. But how do the ants achieve the high temperatures inside their nest? Together with the heat produced by microbial community, a great importance has the metabolic heat produced by ants themselves and the solar heat brought inside in ant bodies. In spring there are thick sunning clusters on the nest surface, the ants actively seek for hot sun-exposed places and heat themselves up to 40 °C. Later they go back into the nest mound where the accumulated heat is dissipated (Zahn 1958). This way the quick temperature hike may happen. The reason for this behaviour is supposed to be a presence of new brood inside the nest (Kipyatkov and Schederova 1990). In summer months the sunning behaviour disappears and ants become negatively phototactic. Not all ants in the colony take part in the sunning, only about one third. Those individuals repeatedly visit warm (sun-exposed) places and a quick increase of body temperature together with metabolic rate (respiration) happens. The metabolic increase is not permanent, 2 days after the sunning event the metabolic rate of “sunning” and “nonsunning” ants were comparable. Our observation with a thermocamera suggests that solar heat accumulated in ant bodies is transferred into the nest mound.

## References

- Apostol A., Moldoveanu T., Sarlea A., Victorin T. (2016) Can red wood ants predict earthquakes? *Journal of Earth Sciences* 2: 1-10
- Berberich G., Berberich M., Grumpe A., Wohler C., Schreiber U. (2013) Early results of three-year monitoring of Red Wood Ants behavioral changes and their possible correlation with earthquake events. *Animals* 3: 63-84
- Bollazzi M., Roces F. (2002) Thermal preference for fungus culturing and brood location by workers of the thatching grass-cutting ant *Acromyrmex heyeri*. *Insectes Sociaux* 49:153–157
- Bonabeau E., Theraulaz G., Deneubourg J.-L., Aron S., Camazine S. (1997) Self-organization in social insects. *Trends in Ecology & Evolution* 12: 188-193
- Brandt D.C. (1980) Is the mound of *Formica polyctena* Foerst. in origin a simulation of a rock? *Oecologia* 44: 281-282
- Brian M.V. (1973) Temperature choice and its relevance to brood survival and caste determination in the ant *Myrmica rubra* L. *Physiological Zoology* 1973 46: 245–252
- Bujok B., Kleinhenz M., Fuchs S., Tautz J. (2002) Hot spots in the bee hive. *Naturwissenschaften* 89: 299-301
- Calabi P., Porter S.D. (1989) Worker longevity in the fire ant, *Solenopsis invicta*, ergonomic considerations of correlations between temperature size and metabolic rates. *Journal of Insect Physiology* 35: 643-650
- Calderone N.W. (1998) Proximate mechanisms of age polyethism in the honey bee, *Apis mellifera* L. *Apidologie (Celle)* 29: 127-158
- Cassill D.L., Tschinkel W.R., Vinson S.B. (2002) Nest complexity, group size and brood rearing in the fire ant, *Solenopsis invicta*. *Insectes Sociaux* 49: 158–163
- Castella G., Chapuisat M., Christe P. (2008) Prophylaxis with resin in wood ants. *Animal Behaviour* 75: 1591–1596
- Challet M., Jost C., Grimall A., et al. (2005) How temperature influences displacements and corpse aggregation behaviors in the ant *Messor sancta*. *Insectes Sociaux* 52: 309–315
- Chen Y.H., Robinson E.J.H. (2015) The relationship between canopy cover and colony size of the wood ant *Formica lugubris*: implications for the thermal effects on a keystone ant species. *PLoS ONE* 9: e116113
- Cherix D., Freitag A., Maeder A. (2006) Fourmis des Bois. Lausanne, Switzerland: Musee de Zoologie. In: Stockan J.A., Robinson E.J.H. (Eds.) *Wood Ant Ecology and Conservation*. Cambridge University Press p. 37 - 50
- Coenen-Staß D. (1985) Zum Verhalten der roten Waldameise, *Formica polyctena* (Hymenoptera, Formicidae) im Klimagradient während der Brutpflege. *Verhandlungen der Deutschen Zoologischen Gesellschaft* 78: 204–112
- Coenen-Staß D., Schaarschmidt B., Lamprecht I. (1980) Temperature distribution and calorimetric determination of heat production in the nest of the wood ant, *Formica polyctena* (Hymenoptera, Formicidae). *Ecology* 61: 238-244
- Dlusskij G.M. (1967) *Muravji roda Formica*. Nauka, Moskva
- Domisch T., Ohashi M., Finér L. et al. (2008) Decomposition of organic matter and nutrient mineralisation in wood ant (*Formica rufa* group) mounds in boreal coniferous forests of different age. *Biology and Fertility of Soils* 44: 539–545

- Domisch T., Finer L., Neuvonen S., Niemelä P., Risch A.C., Kilpeläinen J., Ohashi M., Jurgensen M.F. (2009) Foraging activity and dietary spectrum of wood ants (*Formica rufa* group) and their role in nutrient fluxes in boreal forests. *Ecology Entomology* 34: 369-377
- Ellis S., Robinson E.J.H. (2014) Polydomy in red wood ants. *Insectes Sociaux* 61: 111–122
- Esch H. (1960) Über die Körpertemperaturen und den Wärmehaushalt von *Apis mellifica*. *Zeitschrift für vergleichende Physiologie* 43: 305-335
- Forel A. (1920) *Les fourmis de la Suisse*. Second edition. Imprimerie Cooperative, La Claud de Fernds, Switzerland
- Foster K.R., Wenseleers T., Ratnieks F.L.W. (2006) Kin selection is the key to altruism. *Trends in Ecology & Evolution* 21: 57–60
- Frouz J. (1996) The role of nest moisture in thermoregulation of ant (*Formica polyctena*, Hymenoptera, Formicida) nest. *Biologia, Bratislava* 51: 541 - 547
- Frouz J. (2000) The effect of nest moisture on daily temperature regime in the nests of *Formica polyctena* wood ants. *Insectes Sociaux* 47: 229-235
- Frouz J., Šantrůčková H., Kalčík J. (1997) The effect of wood ants (*Formica polyctena* Foerst.) on the transformation of phosphorus in a spruce plantation. *Pedobiologia* 41: 437–447
- Frouz J., Finer L. (2007) Diurnal and seasonal fluctuations in wood ant (*Formica polyctena*) nest temperature in two geographically distant populations along a south-north gradient. *Insectes Sociaux* 54: 251-259
- Frouz J., Jílková V. (2008) The effect of ants on soil properties and processes (Hymenoptera: Formicidae). *Myrmecological News* 1: 191-199
- Frouz J., Rybníček M., Cudlín P., Chmelíková E. (2008). Influence of the wood ant, *Formica polyctena*, on soil nutrient and the spruce tree growth. *Journal of Applied Entomology* 132: 281-284
- Gobin B., Billen J., Peeters, C. (1999) Policing behaviour towards virgin egg layers in a polygynous ponerine ant. *Animal Behaviour* 58: 1117-1122
- Gordon D.M. (1996) The organization of work in social insect colonies. *Nature* 380: 121-124
- Hamilton W. D. (1964) The genetical evolution of social behaviour. *Journal of Theoretical Biology* 7: 1-16
- Hammond R.L., Bruford M.W., Bourke A.F.G. (2002) Ant workers selfishly bias sex ratios by manipulating female development. *Proceedings of The Royal Society B*, DOI: 10.1098/rspb.2001.1860
- Hannonen M, Sundström L. (2003) Worker nepotism among polygynous ants. *Nature* 421: doi:10.1038/421910a
- Heinrich B. (1981) Energetics of honeybee swarm thermoregulation. *Science* 212: 565-566
- Heinrich B. (1993) *The Hot-blooded Insects. Strategies and Mechanisms of Thermoregulation*. Springer-Verlag Berlin Heidelberg, Berlin
- Heinrich B. (1995) Insect thermoregulation. *Endeavour* 19: 28-33
- Helanterä H., Sundström L. (2007) Worker reproduction in *Formica* ants. *American Naturalist* 170: E14–E25
- Hölldobler B., Wilson E.O. (1990) *The Ants*. Belknap Press, Springer, Berlin
- Horstmann K. (1974) Untersuchungen über den Nahrungserwerb der Waldameisen (*Formica polyctena* Foerster) im Eichenwald. III. Jahresbilanz, *Oecologia* 15: 187-204

- Horstmann K. (1990) Zur Entstehung des Wärmezentrums in Waldameisennestern (*Formica polyctena* Förster, Hymenoptera, Formicidae). Zoologische Beiträge 33: 105–124
- Jílková V. (2015) Wood ants of genus *Formica* as important ecosystem engineers. PhD Thesis, Faculty of Science, Charles University, Prague
- Jílková V., Šebek M., Frouz J. (2012) Mechanisms of pH change in wood ant (*Formica polyctena*) nests. Pedobiologia 55: 247-251
- Jílková V., Frouz J. (2014) Contribution of ant and microbial respiration to CO<sub>2</sub> emission from wood ant (*Formica polyctena*) nests. European Journal of Soil Biology 60: 44-48
- Jílková V., Cajthaml T., Frouz J. (2015) Respiration in wood ant (*Formica aquilonia*) nests as affected by altitudinal and seasonal changes in temperature. Soil Biology & Biochemistry 86: 50-57
- Jílková V., Pícek T., Frouz J. (2015) Seasonal changes in methane and carbon dioxide flux in wood ant (*Formica aquilonia*) nests and the surrounding forest soil. Pedobiologia 58: 7-12
- Jones J.C., Myerscough M.R., Graham S., Oldroyd A.P. (2004) Honeybee nest thermoregulation: Diversity promotes stability. Science 305: 402-404
- Jones J.C., Oldroyd B.P. (2006) Nest Thermoregulation in Social Insects. Advances in insect physiology 33: 153-191
- Kadochová Š. (2011) Thermoregulation of wood ant genus *Formica* on elevation gradient. Diploma thesis, Faculty of Science, Charles University, Prague
- Kadochová Š. and Frouz J. (2013) Thermoregulation strategies in ants in comparison to other social insects, with a focus on *Formica rufa*. F1000Research, 2:280, doi: 10.12688/f1000research.2-280.v1
- Kasimova R.G., Tishin D., Obnosov Y.V., Dlussky G.M., Baksht F.B., Kacimov A.R. (2014). Ant mound as an optimal shape in constructal design: Solar irradiation and circadian brood/fungi-warming sorties. Journal of theoretical biology 355: 21-32
- Keller L., Ross K.G. (1998) Selfish genes: A green beard in the red fire ant. Nature 394: 573-575
- Kilpeläinen J., Punttila P., Finér L., Niemelä P., Domisch T., Jurgensen M.F., Neuvonen S., Ohashi M., Risch A.C., Sundström L. (2008). Distribution of ant species and mounds (*Formica*) in different-aged managed spruce stands in eastern Finland. Journal of Applied Entomology 132: 315–325
- Kilpeläinen, J., Finér L., Neuvonen S., Niemelä P., Domisch T., Risch A.C., Jurgensen M.F., Ohashi M., Sundström L. (2009) Does the mutualism between wood ants (*Formica rufa* group) and *Cinara* aphids affect Norway spruce growth? Forest Ecology and Management 257: 238-243
- Kipyatkov V.E., Schederova S.S. (1990) The endogenous rhythm of queens reproductivity in red wood ants (*Formica* group). Zoologicheskii zhurnal 69: 40-52
- Kleinhenz M., Bujok B., Fuchs S., Tautz J. (2003) Hot bees in empty broodnest cells: heating from within. Journal of Experimental Biology 206: 4217-4231
- Kneitz G. (1964) Untersuchungen zum Aufbau and zur Erhaltung des Nestwärmehaushaltes bei *Formica polyctena* Foerst (Hym, Formicidae). Disertation, University of Würzburg, Würzburg
- Korb J., Linsenmair K.E. (1998) The effect of temperature on the architecture and distribution of *Macrotermes bellicosus* (Isoptera, Macrotermitinae) mounds in different habitats of West African Guinea savanna. Insectes Sociaux 45: 51–65
- Lindauer M. (1954) Temperaturregulierung und Wasserhaushalt im Bienenstaat. Zeitschrift für vergleichende Physiologie 36: 391-432

- Martin A.J. (1980) Vernal thermoregulation in mound nests of *Formica aquilonia* Yarrow, the active heating of brood chambers. *Izvestija Akademii Nauk Estonskoj* 29: 188-197
- Mlota N.J., Tovey C.A., Hua D.L. (2011) Fire ants self-assemble into waterproof rafts to survive floods. *PNAS* 108: 7669–7673
- Muth M. (2012). Untersuchungen zur Wärmespeicherkapazität und zum Wärmetransport der Ameisenart *Formica polyctena* im Zusammenhang mit dem frühjährlichen Aufbau des Nestwärmehaushalts. Diploma Thesis, University of Würzburg, Würzburg
- Neven L.G. (2000) Physiological responses of insects to heat. *Postharvest Biology and Technology* 21: 103-111
- Novgorodova T. (2005) Ant-aphid interactions in multispecies ant communities: Some ecological and ethological aspects. *European Journal of Entomology* 102: 495-501
- Ono M., Igarashi T., Ohno E., Sasaki M. (1995) Unusual thermal defence by a honeybee against mass attack by hornets. *Nature* 337: 334-336
- Oster G.F., Wilson E.O. (1978) *Caste and Ecology in the Social Insects*. Princeton University Press, Princeton
- Otto D. (2005) *Die Roten Waldameisen*. Westarp Wissenschaften, Hohenwarsleben
- Page R.E. Jr, Peng C.Y.-S. (2001) Aging and development in social insects with emphasis on the honey bee, *Apis mellifera* L. *Experimental Gerontology* 36: 695-711
- Penick C.A., Tschinkel W.R. (2008) Thermoregulatory brood transport in the fire ant *Solenopsis invicta*. *Insectes Sociaux* 5: 176–182
- Pokarzhevskij A.D. (1981) The distribution and accumulation of nutrients in nests of ant *Formica polyctena* (Hymenoptera, Formicidae). *Pedobiologia* 21: 117–124
- Porter S.D. (1988) Impact of temperature on colony growth and developmental rates of the ant *Solenopsis invicta*. *Journal of Insect Physiology* 34: 1127–1133
- Porter S.D., Tschinkel W.R. (1987) Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): effects of weather and season. *Environmental Entomology* 16: 802-808
- Punttila P. (1996) Succession, forest fragmentation, and the distribution of wood ants. *Oikos* 75: 291-298
- Ravary F., Lecoutey E., Kaminski G., Châline N., Jaisson P. (2007) Individual experience alone can generate lasting division of labor in ants. *Current Biology* 17: 1306-1312
- Risch A. C., Jürgensen M. F., Page-Dumroese D. S., Schütz M. (2005) The contribution of red wood ants to soil C and N pools and CO<sub>2</sub> emissions in subalpine forests. *Ecology* 86: 419-430
- Robinson G.E. (1992) Regulation of division of labor in insect societies. *Annual Review of Entomology* 37: 637-665
- Robinson E.J.H., Feinerman O., Franks N.R. (2009). Flexible task allocation and the organization of work in ants. *Proceedings of the royal Society B*, doi:10.1098/rspb.2009.1244
- Roces F., Núñez J.A. (1989) Brood translocation and circadian variation of temperature preference in the ant *Campotonus mus*. *Oecologia* 81:33–37
- Rosengren R. (1971) Route fidelity, visual memory and recruitment behaviour in foraging wood ants. *Acta Zoologica Fennica* 133: 1–106



- Rosengren R., Fortelius W., Lindström K., Luther A. (1987) Phenology and causation of nest heating and thermoregulation in red wood ants of the *Formica rufa* group studied in coniferous forest habitats in southern Finland. *Annales Zoologici Fennici* 24: 147-155
- Rosengren, R., Pamilo, P. (1983) The evolution of polygyny and polydomy in mound-building *Formica* ants. *Acta Entomol Fenn* 42: 65-77
- Sakagami Sh. F. (1954) Occurrence of an aggressive behaviour in queenless hives, with considerations on the social organization of honeybee. *Insectes Sociaux* 1: 331–343
- Seeley T.D., Heinrich B. (1981) Regulation of temperature in the nest of social insects In: Heinrich B. (Ed). *Insect Thermoregulation*, John Wiley and Sons, pp. 160–234
- Seeley T.D. (2009) *The wisdom of the hive: the social physiology of honey bee colonies*. Cambridge University Press, Massachusetts
- Schwander T., Rosset H., Chapuisat M. (2005) Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. *Behavioral Ecology and Sociobiology* 59: 215–221
- Sorvari J. (2013). Proximity to the forest edge affects the production of sexual offspring and colony survival in the red wood ant *Formica aquilonia* in forest clear-cuts. *Scandinavian Journal of Forest Restoration* 28: 451-455
- Starks P.T., Johnson R.N. Siegel A.J., Decelle M.M. (2004) Heat shielding: a task for youngsters. *Behavioral Ecology* 16: 128-132
- Steiner A. (1924) Über den sozialen Wärmehaushalt der Waldameise (*Formica rufa*). *Zeitschrift für vergleichende Physiologie* 2: 23-56
- Stockan J.A., Robinson E.J.H. (2016) *Wood Ant Ecology and Conservation*. Cambridge University Press, Cambridge
- Tamarri V., Castracani C., Grasso D.A., Visicchio R., Le Moli F., Mori A. (2009) The defensive behaviour of two *Formica* slave-ant species: coevolutionary implications with their parasite *Polyergus rufescens* (Hymenoptera, Formicidae). *Italian Journal of Zoology* 76: 229-238
- Theraulaz G., Bonabeau E., Deneubourg J.-L. (1998) Response threshold reinforcements and division of labour in insect societies. *Proceedings of The Royal Society B*. 265: 327-332
- Trivers R.L., Hare H. (1976) Haplodiploidy and the Evolution of the Social Insects. *Science* 191: 249-263
- Wassman E. (1915) *Das Gesellschaftsleben der Ameisen*. Aschendorf, Münster
- Wilson E.O. (1971) *The insect societies*. Belknap Press of Harvard University Press, Massachusetts
- Zahn M. (1958) Temperatursinn, Wärmehaushalt und Bauweise der rote Waldameisen (*Formica rufa* L.). *Zoologische Beiträge* 3: 127-194
- Zakharov A.A. (1980) Observer ants: storers of foraging area information in *Formica rufa* L. Formicidae, Hymenoptera). *Insectes Sociaux* 27: 203–211
- Žďárek J. (2013) *Hmyzí rodiny a státy*. Academia, Praha

## Enclosed papers

### Paper 1

**Kadochová Š., Frouz J.** (2014) Red wood ants *Formica polyctena* switch off active thermoregulation of the nest in autumn. *Insectes Sociaux* 61: 297-306

Contribution of Štěpánka Kadochová = 85%

An important share on formulation of the research question, conduction of field measurements and data collection, statistic data processing, writting the manuscript.

### Paper 2

**Kadochová Š., Frouz J., Tószögyová A** (submitted 2017) Seasonal changes in clustering and phototaxis, two important aspects of sun basking behaviour in red wood ants (*Formica polyctena*). *Journal of Insect Behavior*, accepted for major revision

Contribution of Štěpánka Kadochová = 70%

A significant share on the formulation of research question and experimental set up, realization of field measurements and data collection, conduction of the shading experiment, evaluation of IR data, writting of the manuscript.

### Paper 3

**Kadochová Š., Frouz J., Roces F.** (2017) Sun basking in red wood ants *Formica polyctena* (Hymenoptera, Formicidae): Individual behaviour and temperature-dependent respiration rates. *PLoS ONE* doi:10.1371/journal.pone.0170570

Contribution of Štěpánka Kadochová = 75%

An important share on the formulation of research question and experimental set up, design of the laboratory experiment, execution of tests and respirometric measurement, evaluation of videos, statistic data processing, writing of the manuscript.

In behalf of all co-authors

Jan Frouz

