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ORIGINAL ARTICLE

Temperature differences associated with colour do not determine where the acorn ant *Temnothorax crassispinus* (Hymenoptera: Formicidae) chooses to nest

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Abstract. Temperature is an important factor for invertebrates. Social insects build nests, which along with their ability to thermoregulate, provide shelter from extreme temperatures. However, for many species of ants the most common method of controlling the temperature inside a nest is to choose a suitable nest site. During a field experiment, the choice of nest site by the acorn ant *Temnothorax crassispinus*, a species which lives in coniferous and mixed forests, was studied. It typically occupies ephemeral nest sites and can move to a new nest site several times in one season. It was predicted that in early spring, dark coloured nest sites would be warmer and thus more frequently occupied by ant colonies. Contrary to this prediction, no difference was recorded in the frequency with which dark and lighter coloured nests were occupied. However, also in contrast to the prediction, in forest in early spring the difference in temperature inside different coloured nests was small. Thus, other features of nests, e.g., volume of cavity, are probably more important in determining nest site selection by this ant.

INTRODUCTION

Body temperature, behaviour and development of invertebrates are dependent on the temperature of the environment (Cossins & Bowler, 1987). This is also true of social insects, in which temperature can affect brood development and consequently colony growth. Thus, many social insects build nests that provide shelter and protection from extreme temperatures (Jones & Oldroyd, 2007; Blüthgen & Feldhaar, 2010). In addition, many social insects can regulate the temperature inside their nests, both actively and passively. The former by individuals changing nest temperature by incubating, fanning and evaporating water, which is well known for bees and wasps, and the latter by nest orientation, nest architecture and nest site selection, as reported for different groups of social insects, including ants. In ants, the most common method of controlling temperature in nests is probably moving to more suitable nest sites (Jones & Oldroyd, 2007).

Many species of ants do not construct nests, but utilize available cavities, as nest construction is costly in terms of time and energy (Mikheyev & Tschinkel, 2004; McGlynn, 2012). Ants of the genus *Temnothorax* typically occupy ephemeral nest sites and have to find and move to new nest sites even several times in one season (Herbers, 1989; Herbers & Johnson, 2007). For such species, selection of nest site is crucial. These ants are used in numerous studies on nest site selection. For example, these ants prefer nests with narrow entrances, which are easier to defend (Pratt & Pierce, 2001; Franks et al., 2003; Pratt, 2010), and with bigger cavities (Mitrus, 2015).

The acorn ant *Temnothorax crassispinus* (Karawajew, 1926) is present throughout Western and Central Europe (Czechowski et al., 2012; Seifert, 2018). Workers of this ant are small, 2–4 mm in length; colonies typically number from a few dozen to about 200 workers (Czechowski et al., 2012; Seifert, 2018). This ant mostly inhabits cavities in acorns, small sticks and logs in the litter layer (Białas et al., 2011; Czechowski et al., 2012; Seifert, 2018). Colonies of this ant are frequently found in old acorns, which typically have a dark brown colouration (personal observation). Because of their dark colour, such acorns should be more heated by the sun. The ant lives in coniferous and mixed forests (Czechowski et al., 2012; Seifert, 2018). Thus, in the spring before the leaves of trees start to block out sunlight it could be advantageous to choose dark coloured nest sites, which warm up faster. In contrast to the majority of ants living in the temperate zone, which spend winter underground, acorn ants of the genus *Temnothorax* overwinter in nests aboveground (Herbers, 1989; Herbers & Johnson, 2007). Berman et al. (2010) suggest that overwintering in aboveground nests enables this ant to become active earlier in spring than other species of ants. Thus, inhabiting warmer nest sites in the spring would be an additional benefit.

The aim of this study was to check if under natural conditions the acorn ant *T. crassispinus* chooses dark coloured nest sites. For this a field experiment using artificial nests of different colours was carried out. The prediction was that the darker nests would be considerably warmer, and thus preferred by this ant in the early spring.

MATERIAL AND METHODS

The field experiment was carried out in a beech-pine forest with a few oaks (Poland, Opole district, 50.6247N, 18.1090E), in an area where a high density of colonies of *Temnothorax crassispinus* were recorded during previous research. The soil in the area is covered by fallen beech and oak leaves, pine needles, twigs, acorns and pine cones; only about 5% of the soil surface is uncovered. Vegetation is scarce (cover of herbaceous plant layer: < 5%; cover of moss and lichen: < 1%), thus during the experiment the artificial nests (see below) were not shaded by herbaceous plants, but some of them were periodically partially covered by dry, fallen leaves. During the experiment, artificial nests made of blocks of beech wood with nest cavities cut into them are readily accepted by *Temnothorax* ants (cf. Foitzik et al., 2003, Mitrus, 2015, 2019). Each woodblock used in the experiment (size: approximately 7.0 cm × 1.8 cm × 1.8 cm) was drilled lengthwise to form a 4 mm hole, which was tightly closed at one end with a beech plug. After calculating the reduction in the length of the drilled cavity due to the plug, the final cavity volume was approximately 750 mm^3. The hole at the other end was reduced by 50% using a beech splinter (Mitrus, 2019), as ants prefer cavities with narrow entrances (see Introduction). Nest sites were randomly divided into two groups with those in the first group painted with brown coloured lacquer and the second with transparent lacquer. The whole surface of each nest was painted, except for an area roughly 1 cm wide around the entrance (cf. Fig. 1). Acrylic water-based lacquer was used (Eco Revolution, producer: Chemmot Poland; environmentally friendly lacquers): brown chocolate colour lacquer (‘dark’ nests: RAL colour standard 8017 is similar to the colour of old acorns), and transparent lacquer (these nests were bright brown = ‘light’ nests). Then, pairs of ‘dark’ and ‘light’ nests were joined by a piece of wood about 5 mm thick (Fig. 1) so the distance between the nest sites was approximately 5 mm and between entrances approximately 2.5 cm.

In this study 40 pairs of nests were used and the distance between neighbouring pairs of nests was approximately 75 cm. They were located in the forest, 5–8 m from edge of the forest. Pairs of nests were attached to sticks approximately 16 cm long, poked into the ground. In addition, pairs of nest sites were used to measure temperature inside the nest cavity. These nests had an additional entrance tunnel measuring 4 mm wide drilled in the side of the block so that a data logger sensor could be located just inside the cavity. This tunnel was then sealed, and the entrance to the nest closed with metal netting. Thus, the nests with data logger sensors were empty during the experiment, that is, ant colonies or other invertebrates were not able to inhabit the chambers. For this experiment previously calibrated AZ 88128 data loggers were used. Nests with data logger sensors were placed in the centre of the experimental plot (approximately 6.5 m from edge of the forest) and just at the edge of the forest. The temperature was monitored at 5 min intervals, but due to technical problems temperature data was only available until 28 March.

The nests were placed in the experimental plot on 1 February 2017 when air temperature was about –2°C and there was little snow in the area. On 3 April the pairs of nests were collected,

Fig. 1. Photograph of a pair of artificial nests used in this study. Forty such pairs of artificial nests, which differed in colour, were placed in forest on 1 February 2017 and collected about two months later. Such nests made of block of wood are readily accepted by acorn ants of the genus *Temnothorax*.

disconnected from each other, placed to separate bags and transported to the laboratory. In the laboratory, they were carefully opened, the ants captured with an aspirator and counted. In the case of a nest cavity containing only workers (i.e., where no eggs or pupae were found), they were classified as ‘containing only a few individuals’.

Data analysis: Chi-square test was used to test if ‘dark’ and ‘light’ coloured nests were inhabited in similar proportions. Numbers of workers in ‘dark’ vs. ‘light’ coloured nests with queens, were compared using Student *t* test; assumptions of normality and homogeneity of variance were checked prior to the analysis. This was done using the software package Statistica, ver. 13 (Dell Inc., 2016). The threshold for significance was $P = 0.05$ throughout. All probability values shown are two-tailed.

RESULTS

In early April 2017, *Temnothorax crassispinus* ants were recorded in 71 of the 80 nests, thus only nine nests were empty (five ‘dark’ and four ‘light’) (Fig. 2). In 32 of these nests only workers were recorded (small groups of workers, no queens, no brood) with 1–10 (mean 5.4, N = 19) and 1–14 workers (mean 6.8, N = 13) in ‘dark’ and ‘light’ coloured nests, respectively. These results were not included in subsequent analyses. For colonies in ‘dark’ coloured nests, two contained no brood with one with and one without a queen. Similarly, colonies in ‘light’ coloured nests, one without and one with a queen contained no brood. In the other colonies eggs or eggs and pupae were present.

Contrary to the prediction, the frequency with which *T. crassispinus* was recorded in ‘dark’ coloured nests was not significantly different from that recorded in ‘light’ coloured ones ($\chi^2 = 2.45$, $df = 1$, $P = 0.12$; cf. Fig. 2). Only in one case, in both joined nest sites, colonies with queen were found: with nine workers in the ‘dark’ nest and with 135 workers in the ‘light’ coloured nest. For the colonies where queens were present, there was no difference in the number of workers in ‘dark’ and ‘light’ coloured nests (for ‘dark’ nests: 9–128, mean 119.7, N = 5, for ‘light’ ones: 5–223, mean 115.5, N = 16; *t* Student test, $t = 1.18$, $df = 19$, $P = 0.25$).

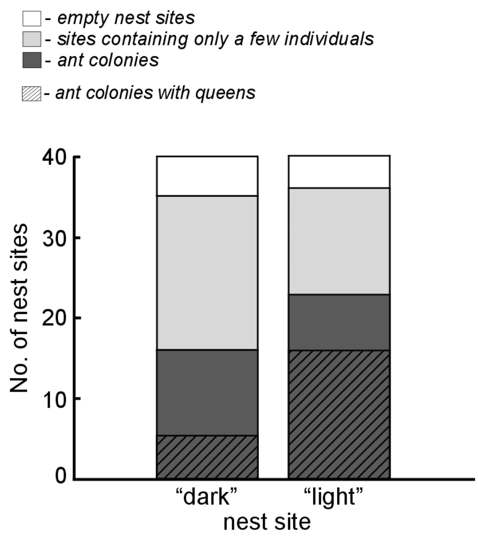

Fig. 2. The number of the 'dark' and 'light' coloured artificial nests in which colonies or a few individuals without brood, or no *Temnothorax crassispinus* ants were recorded. In addition, the shaded areas of the columns indicate the number of artificial nests with ant colonies with queens.

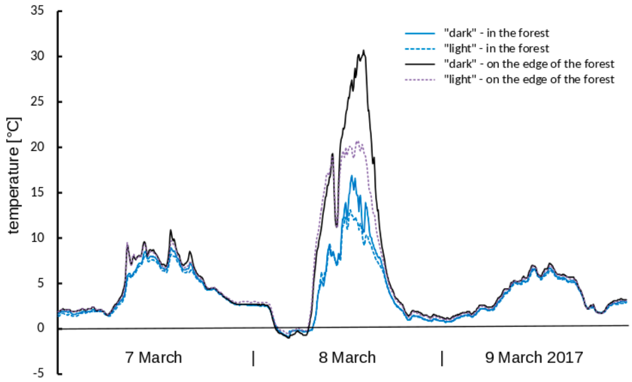

Fig. 3. Changes in temperature recorded inside cavities in woodblocks. Temperature was monitored using data loggers, at 5 min intervals. The pairs of nests ('dark' and 'light' coloured; cf. Fig. 1) were placed in the experimental plot (approximately 6.5 m from the edge of the forest) and just at the edge of the forest. Data for three successive days are presented: it was sunny on March 8th and cloudy on March 9th.

Nests located in the forest were generally cooler than those at the forest edge that were more exposed to direct sunlight (Table 1). At night and in cloudy weather, the recorded difference in temperature between a pair of 'dark' and 'light' coloured nests was small, typically +/– 0.2°C (see Fig 3, March 9th). However, on sunny days, 'dark' nests were warmer, even considerably (see Fig. 3, March 8th). The biggest difference in temperature recorded in the cavities of 'dark' and 'light' nests located in forest was 5.8°C (mean difference for the period analysed: 0.22°C) and at the edge of the forest, 16.6°C (mean difference: 0.92°C). Differences in temperature between nests located in the forest compared to the ones on the edge of the forest were a maximum 9.0°C (mean: 2.47°C), and a maximum 11.2°C (mean: 1.72°C), for 'dark' and 'light' nest sites, respectively.

DISCUSSION

Most of artificial nests were occupied by the acorn ant *Temnothorax crassispinus* in this study, which indicates that nest sites are a limited resource for this ant (cf. Foitzik & Heinze, 1998). Only in one case both nests of a pair of nests were occupied by a colony with a queen. Colonies of the ant species could be polydomous (Strätz & Heinze, 2004), i.e., a colony simultaneously occupies several nests. Thus, it is probable that workers in neighbouring (i.e., joined) nests belonged to the same colony.

Contrary to the prediction that in early spring colonies of the ant *T. crassispinus* would prefer 'dark' coloured nests, no difference was found in the frequency in which 'dark' and 'light' coloured nests were occupied. However, the prediction that in early spring before the leaves of the trees block out sunlight there would be a significant difference in temperature inside nests that differ in colour, as it is well known that dark coloured substrates absorb more solar energy than light coloured ones (Cossins & Bowler, 1987), was also not confirmed. In the nests located in the forest only a small and temporary difference in temperature was recorded (see Table 1 and Fig. 3). Although it is well known that trees can protect environments from climate extremes (e.g., Suggitt et al., 2011; von Arx et al., 2013) the prediction was that in early spring there would be a noticeable difference in the temperature in 'dark' and 'light' coloured nests. Thus, based on the results of this study the benefits of choosing 'dark' coloured nests would appear to be either too small or the ant is unable to compare nest sites in this regard because the temperature can be variable.

Temperature is important for social insects, because it affects the rate of brood and colony growth. The North American acorn ant *T. curvispinosus* has a high egg-laying rate at 26°C and the larval development ceases below 23°C (Penick et al., 2017), thus acorn ants that select warm nest sites would be at an advantage. Karlik et al. (2016) demonstrate that to ensure the best conditions for brood development, acorn ants use different locations within a nest, even inside a cavity as small as an acorn: when they are heated from above, workers move brood to the warm, upper half of the acorn, but the distribution of workers does not differ significantly between the warm and cool parts of nest (Karlik et al., 2016).

Colour of the nest did not affect whether it was inhabited or not, but the majority were inhabited by acorn ants and

Table 1. Temperatures recorded in artificial nests inside dark and light-coloured blocks of wood (Fig. 1), 1–27 March 2017. These nests were used in a field study of the choice of nests by the ant *Temnothorax crassispinus*. Nests were located in an experimental plot and at the edge of the forest; those at the experimental plot – approximately 6.5 m from the edge. Temperature was monitored using data loggers at 5 min intervals.

	Nest colour	mean/median	min–max	quartiles
Experimental plot	dark	+5.95/+5.3°C	–4.2–+29.6°C	+2.6–+8.6°C
	light	+5.73/+5.3°C	–4.2–+23.8°C	+2.5–+8.4°C
Edge of forest	dark	+8.42/+7.2°C	–4.7–+38.6°C	+3.1–+11.5°C
	light	+7.45/+6.0°C	–4.6–+35.0°C	+2.9–+10.1°C

the effect of forest in buffering temperatures was bigger than predicted. Soil temperature and moisture are the most important factors affecting the distribution of ants (Seifert, 2017). The maximum and mean soil temperatures describing the niche dimensions of *T. crassispinus* are among the lowest recorded for the 86 species of ants in Central Europe (see Seifert, 2017). So, it is possible that this species prefers cool places. In Opole, the monthly average air temperature in March in the period 1951 to 2000 was 3.2°C (Woś, 2010), but in March 2017 it was much warmer with an average of 7.0°C (Dane meteorologiczne, 2020). Therefore, the weather conditions could have affected the results: higher than usual air temperature after overwintering could have affected the search for cooler places for nesting. However, temperature in spring varies between years. Thus, small difference in temperature between 'dark' and 'light' coloured nests, rather than high mean temperature in March 2007, is possibly the reason why *T. crassispinus* ant colonies inhabited these nest sites with the same frequency. As the ongoing process of climate change predicts higher temperatures (Christensen et al., 2013), it could lead to changes in behaviour and habitat selection by some ants, including species of the genus *Temnothorax*. To determinate the nest selection by the ant, laboratory experiments on thermal preferences of the workers and broods are recommended, e.g., do colonies containing brood vs. without broods prefer similar temperatures and is their thermal preference similar in different seasons (i.e., early and late spring and summer).

Selection of warm or cool nest sites by ants could be affected by factors not studied. For example, if food is limited, colonies of the fire ants (*Solenopsis invicta*) prefer cool temperatures (Porter & Tschinkel, 1993) and the unusual high temperatures in March 2007 could have also affected other species and consequently the availability of food during this experiment. In addition, as nest cavities are a limited resource for acorn ants, other features of these cavities (e.g., volume and entrance size) could be more important for these ants, but in this experiment all nests were similar. That the acorn ant *T. crassispinus* colonies frequently inhabit dark acorns, could simply be because such acorns provide a suitable cavity for a colony.

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