Nesting behaviour of the paper wasp *Polistes dominula* – with special focus on thermoregulatory mechanisms

....

Nistverhalten der Feldwespe *Polistes dominula* – mit besonderem Augenmerk auf thermoregulatorische Mechanismen

Doctoral thesis for a doctoral degree at the Graduate School of Life Sciences,
Julius-Maximilians-Universität Würzburg

Section: Integrative Biology

submitted by

Nicole Höcherl

from

Wörth an der Donau

Würzburg 2015

Submitted on: ....

Office stamp

Members of the *Promotionskomitee*:

Chairperson: Prof. Dr. Thomas Dandekar

Primary Supervisor: Prof. Dr. Jürgen Tautz

Supervisor (Second): PD Dr. Johannes Spaethe

Supervisor (Third): Prof. Dr. Stefano Turillazzi

Date of Public Defence: ……………………………………………..………

Date of receipt of Certificates: ……………………………………….…..

Affidavit

I hereby confirm that my thesis entitled '**Nesting behaviour of *Polistes dominula* – with special focus on thermoregulatory mechanisms**' is the result of my own work. I did not receive any help or support from commercial consultants. All sources and/or materials applied are listed and specified in the thesis.

Furthermore, I confirm that this thesis is has not yet been submitted as part of another examination process neither in identical nor similar form.

Würzburg, 22.12.2015……………………………………………

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation '**Nesting behaviour of *Polistes dominula* – with special focus on thermoregulatory mechanisms**' eigenständig, d.h. insbesondere selbstständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Würzburg, 22.12.2015……………………………………………

The Wasp to the Butterfly:

“Non qui fuerimus,
sed qui nunc simus vide!“

“Do not consider what we were,
but what we are!“

„Schau nicht nach dem, was wir waren,
sondern auf das was wir sind!“

from

Phaedrus, Liber Fabularum, Appendix Perottina 31:

Papilio et Vespa (The Butterfly and the Wasp; Der Schmetterling und die Wespe)

TABLE OF CONTENT

LIST OF USED ABBREVIATIONS AND SYMBOLS

%	“percent”
°C	centigrade
±	plus-minus sign
≈	approximately
Σ	“sigma sign”
©	copyright
®	registered trademark symbol
™	unregistered trademark
</≤	less-than/ less-than-or-equal sign
>/≥	greater-than /greater-than-or-equal sign
am	“ante meridiem”
ca.	circa/about/approximately
cm	centimetre
CO_2	carbon dioxide
e. g.	“exempli gratia”/for example/ for instance
etc.	“et cetera”
Fig.	figure
d	day (s)
g	gram
h	hour (s)
i. e.	“id est”/in other words
km	kilometre
min	minute (s)
no.	number
O_2	oxygen
pm	“post meridiem”
s	second (s)

SUMMARY

Wasps of the genus *Polistes* comprise over 200 species and are nearly cosmopolitan. They show a lack of physiological caste differentiation and are therefore considered as primitively eusocial. Furthermore, paper wasps are placed between the solitary living Eumenidae and the highly social organized Vespinae. Hence, they are often called a "key genus" for understanding the evolution of sociality. Particularly, *Polistes dominula,* with its small easy manageable nests and its frequent occurrence and wide distribution range is often the subject of studies.

In Europe, the invasion of this species into northern regions is on the rise. Since little was known about the nesting behaviour of *P. dominula* in Central Europe, the **basic principles about nesting** were investigated in Würzburg, Germany (latitude 49°) by conducting a comprehensive field-study spanning three consecutive years. Furthermore, the **thermoregulation of individual wasps** in their natural habitat had not yet been investigated in detail. Therefore, their ability to respond to external hazards with elevated thorax temperatures was tested. In addition, different types of **nest thermoregulation** were investigated using modern methods such as infrared thermography and temperature data logger.

In the present work, the investigation of **basic nesting principles** revealed that foundress groups (1-4 foundresses) and nests are smaller and that the nesting season is shorter in the Würzburg area than in other regions. The mean size of newly founded nests was 83 cells and the average nesting season was around 4.6 months. The queens neither preferred single (54%) nor multiple founding (46%) in this study. The major benefit of multiple founding is an increased rate of survival. During the three years of observation, only 47% of single-foundress colonies survived, whereas 100% of colonies that were built by more than two queens, survived. However, an influence of the number of foundresses on the productivity of colonies in terms of number of cells

and pupae per nest has not shown up. However, the length of the nesting season as well as the nest sizes varied strongly depending on the climatic conditions of the preceding winter during the three consecutive years.

In order to investigate the **thermoregulatory mechanisms of individual adult *P. dominula* wasps**, I presented artificial threats by applying smoke or carbon dioxide simulating fire and predator attacks, respectively, and monitored the thorax temperature of wasps on the nest using infrared thermography. The results clearly revealed that *P. dominula* workers recognized smoke and CO_2 and reacted almost instantaneously and simultaneously with an increase of their thorax temperature. The maximal thorax temperature was reached about 65 s after the application of both stressors, but subsequently the wasps showed a different behaviour pattern. They responded to a longer application of smoke with moving to the exit and fled, whereas in case of CO_2 the wasps started flying and circling the nest without trying to escape. No rise of the thorax temperature was detectable after an air blast was applied or in wasps resting on the nest. Additionally, the thorax temperatures of queens were investigated during dominance battles. I found that the thorax temperature of the dominant queens rose up to 5°C compared to that of subordinate queens that attacked the former.

The study of **active mechanisms for nest thermoregulation** revealed no brood incubation or clustering behaviour of *P. dominula*. Furthermore, I found out that wing fanning for cooling the nest was almost undetectable (4 documented cases). However, I could convincingly record that water evaporation is most effective for nest cooling. By the direct comparison of active (with brood and adults) and non-active (without brood and adults) nests, the start of cooling by water evaporation was detected above maximum outside temperatures of 25°C or at nest temperatures above 35°C. The powerful role of water in nest cooling was manifested by an average decrease of temperature of a single cell of about 8°C and a mean duration of 7 min until the cell reached again its initial temperature. The investigation of **passive thermoregulatory mechanisms** revealed that

the nest site choice as well as nest orientation appears to be essential for *P. dominula* wasps. Furthermore, I was able to show that the architecture of the nests plays an important role. Based on the presented results, it can be assumed that the vertical orientation of cells helps maintaining the warmth of nests during the night, whereas the pedicel assists in cooling the nest during the day.

The results about **basic nesting principles** imply that *P. dominula* is remarkably flexible and reacts very quickly to outside conditions. This adaptation could be one explanation for the rapid spread of this species into colder areas. The observations made by the investigation of **thermoregulatory mechanisms of the individual wasp** provide evidence that *P. dominula* is able to heat up its thorax and that thermoregulation is employed in escape and defence reactions. The study of **nest thermoregulation** has revealed that *P. dominula* wasps regulate the temperature of their nest actively by cooling by evaporation of water and passively by a careful site selection and the architecture of their nests.

Taken as a whole, I could show that *P. dominula* wasps are very flexible in their nesting behaviour and that nesting itself is strongly linked to temperature conditions. Although these insects are already able to heat up their flight muscles, they do not utilize this ability for an active incubation of their brood. It remains to be verified whether there is an evolutionary link between the heating up of the flight muscles in response to a defence situation, as shown for *P. dominula* in this work, and the heating up of the wing muscles for incubating the brood in higher eusocial insects.

ZUSAMMENFASSUNG

Die Wespen-Gattung *Polistes* ist mit ihren über 200 Arten nahezu auf der ganzen Welt vertreten. Da die physiologischen Unterschiede zwischen den Kasten fehlen, werden sie als primitiv eusozial eingestuft. Des Weiteren werden sie zwischen den solitär lebenden Eumenidae und den hoch eusozialen Vespinae eingeordnet. Sie werden daher oft als „Schlüssel-Gattung“ für das Verständnis der Evolution von Sozialität bezeichnet. Insbesondere *Polistes dominula* (Haus-Feldwespe) wird aufgrund der kleinen einfach zu handhabenden Nester, dem häufigen Vorkommen und der weiten Verbreitung vielfach für Studien genutzt.

In Europa ist diese Wespenart auf dem Vormarsch in nördlichere Regionen. Bisher war kaum etwas über das Nistverhalten von *P. dominula* in Zentraleuropa bekannt. Daher wurde eine umfassende, drei aufeinanderfolgende Jahre andauernde Freilandstudie zu den **Grundlagen des Nistverhaltens** in Würzburg (Deutschland, Breitengrad 49°) durchgeführt. Auch die **Thermoregulation der Einzeltiere** wurde noch nie im Detail erforscht. Daher wurde ein Experiment durchgeführt, das aufzeigen sollte, ob diese Tiere die Fähigkeit besitzen, mit erhöhten Thoraxtemperaturen auf Gefahr zu reagieren. Zusätzlich kamen neuere Methoden wie die Infrarot-Thermographie und Temperaturdatenlogger zum Einsatz, um die verschiedenen Arten der **Nestthermoregulation** zu untersuchen.

In der vorliegenden Arbeit über die **Grundlagen des Nistverhaltens** zeigte sich, dass im Vergleich zu anderen Regionen in Würzburg sowohl die Gruppengröße der Nestgründerinnen (1-4 Gründerinnen) als auch die Nester an sich kleiner sind (≈ 83 Zellen) und die Nestsaison kürzer (≈ 4,6 Monate). Die Königinnen bevorzugten weder die Gründung des Nestes allein (54%) noch zusammen mit mehreren Königinnen (46%). Der größte Vorteil einer Gründung der Nester durch mehrere Königinnen liegt in einer erhöhten Überlebensrate der Nester. In der drei Jahre andauernden Studie überlebten nur 47% der Nester, die von einer Königin gegründet wurden, während 100% der Völker, die von mehr als zwei Königinnen gegründet wurden, überlebten.

Es konnte jedoch kein Einfluss der Anzahl an Gründerinnen auf die Produktivität (bezüglich der Anzahl von Zellen und Puppen) der Völker festgestellt werden. Allerdings variierten Saisonlängen und Nestgrößen stark in Abhängigkeit der klimatischen Bedingungen des vorangegangenen Winters in den drei aufeinanderfolgenden Jahren.

Zur Untersuchung der **thermoregulatorischen Mechanismen der adulten Tiere** setzte ich künstliche Bedrohungen in Form von Rauch und Kohlendioxid ein, um entweder ein Feuer oder einen Raubtierangriff zu simulieren. Die Thoraxtemperaturen der auf dem Nest sitzenden Feldwespen wurde zeitgleich mit einer Thermokamera überwacht. Die Ergebnisse belegen eindeutig, dass *P. dominula*-Arbeiterinnen Rauch und CO_2 wahrnehmen und beinahe unverzüglich und zeitgleich mit einer Erhöhung der Thoraxtemperatur reagieren. Nach der Applikation der beiden Stressoren war die maximale Temperatur nach durchschnittlich 65 s erreicht, allerdings zeigten die Wespen unterschiedliche Verhaltensmuster. Auf eine längere Rauchapplikation reagierten sie mit Flucht, während sie im Fall von CO_2 fliegend das Nest umkreisten, ohne zu fliehen. Nach der Gabe eines Luftstoßes oder bei ruhenden Wespen war kein Anstieg der Thoraxtemperatur nachweisbar. Zusätzlich wurden die Thoraxtemperaturen von Königinnen bei Dominanzkämpfen untersucht. Ich verzeichnete einen Anstieg der Thoraxtemperatur der dominanten Königin um bis zu 5°C im Vergleich zu der Temperatur der untergeordneten Königin, die die dominante angriff.

Die Studie der **aktiven Mechanismen der Nestthermoregulation** belegte, dass bei *P. dominula* kein Heizen der Brut oder „Clustern“ stattfindet. Des Weiteren war Fächeln zur Kühlung des Nestes so gut wie nicht feststellbar (4 dokumentierte Fälle). Allerdings war ich in der Lage nachzuweisen, dass die Verdunstung von Wasser für die Kühlung des Nestes sehr effektiv ist. Durch den direkten Vergleich von aktiven (mit Brut und adulten Tieren) und nicht-aktiven (ohne Brut und adulten Tieren) Nestern konnte der Beginn des Kühlens bei einer maximalen Außentemperatur von über 25°C oder einer Nesttemperatur von über 35°C ermittelt werden. Die wichtige Rolle, die Wasser für die Nestkühlung spielt, zeigte sich zum Einen durch die mittlere

Abkühlung einer einzelnen Zelle von ca. 8°C und zum Anderen durch eine durchschnittliche Dauer von 7 min, bis die Zelle wieder ihre Ausgangstemperatur erreichte. Die Untersuchung der **passiven Mechanismen zur Nestthermoregulation** zeigte, dass sowohl die Wahl des Nistplatzes als auch die Orientierung des Nestes für die Haus-Feldwespe essentiell ist. Darüber hinaus war ich in der Lage nachzuweisen, dass die Architektur des Nestes eine entscheidende Rolle spielt. Auf der Grundlage der vorgestellten Ergebnisse kann angenommen werden, dass die nach unten ausgerichteten Zellen helfen, das Nest nachts zu wärmen, während der Stiel des Nestes hilft, das Nest tagsüber zu kühlen.

Die Ergebnisse zu den **Grundlagen des Nistverhaltens** bedeuten, dass *P. dominula* außergewöhnlich flexibel ist und sehr schnell auf äußere Bedingungen reagiert. Diese Anpassung könnte eine Erklärung für die schnelle Ausbreitung dieser Art in kühlere Regionen sein. Die Untersuchung der **thermoregulatorischen Mechanismen des Einzeltieres** hat bewiesen, dass Wespen der Art *P. dominula* in der Lage sind, ihre Flügelmuskulatur aufzuheizen und dass Thermoregulation ein Teil von Flucht- und Abwehrreaktionen ist. Die Untersuchung der **Nestthermoregulation** offenbarte, dass *P. dominula* Wespen die Temperatur ihrer Nester aktiv über die Kühlung durch Wasserverdunstung und passiv durch eine sehr sorgfältige Wahl des Nistplatzes und die Architektur des Nestes beeinflussen.

Insgesamt konnte ich nachweisen, dass Wespen der Art *P. dominula* äußerst flexibel bezüglich ihres Nistverhaltens sind und dass dieses sehr stark mit den Temperaturbedingungen verknüpft ist. Auch wenn diese Insekten bereits in der Lage sind, ihre Flügelmuskulatur in einer Abwehrreaktion aufzuheizen, so nutzen sie diese Fähigkeit nicht für ein aktives Heizen ihrer Brut. Es bleibt also nachzuweisen, ob es einen evolutionären Zusammenhang zwischen dem Aufheizen der Flügelmuskulatur im Zuge einer Abwehrhandlung (wie für *P. dominula* in der vorliegenden Arbeit gezeigt) und dem Aufheizen der Flügelmuskulatur für das aktive Heizen der Brut (bei höher eusozialen Insekten) gibt.

1. INTRODUCTION

1.1 POLISTES DOMINULA – A MODEL ORGANISM FOR THE EVOLUTION OF SOCIALITY

Within the insect family tree, paper wasps of the genus *Polistes* belong to the apocrita **(Fig. 1)** and are one of the species-richest groups of social wasps (Santos et al. 2015). They involve over 200 species and can be found on six continents (Carpenter 1996, Pardi 1996). *Polistes* are often called a "key genus" for understanding the evolution of sociality, because these paper wasps are placed between the solitary living Eumenidae and the highly social organized Vespinae, like yellow jackets (West-Eberhard 1969, Shakarad and Gadagkar 1995, Jandt et al. 2014).

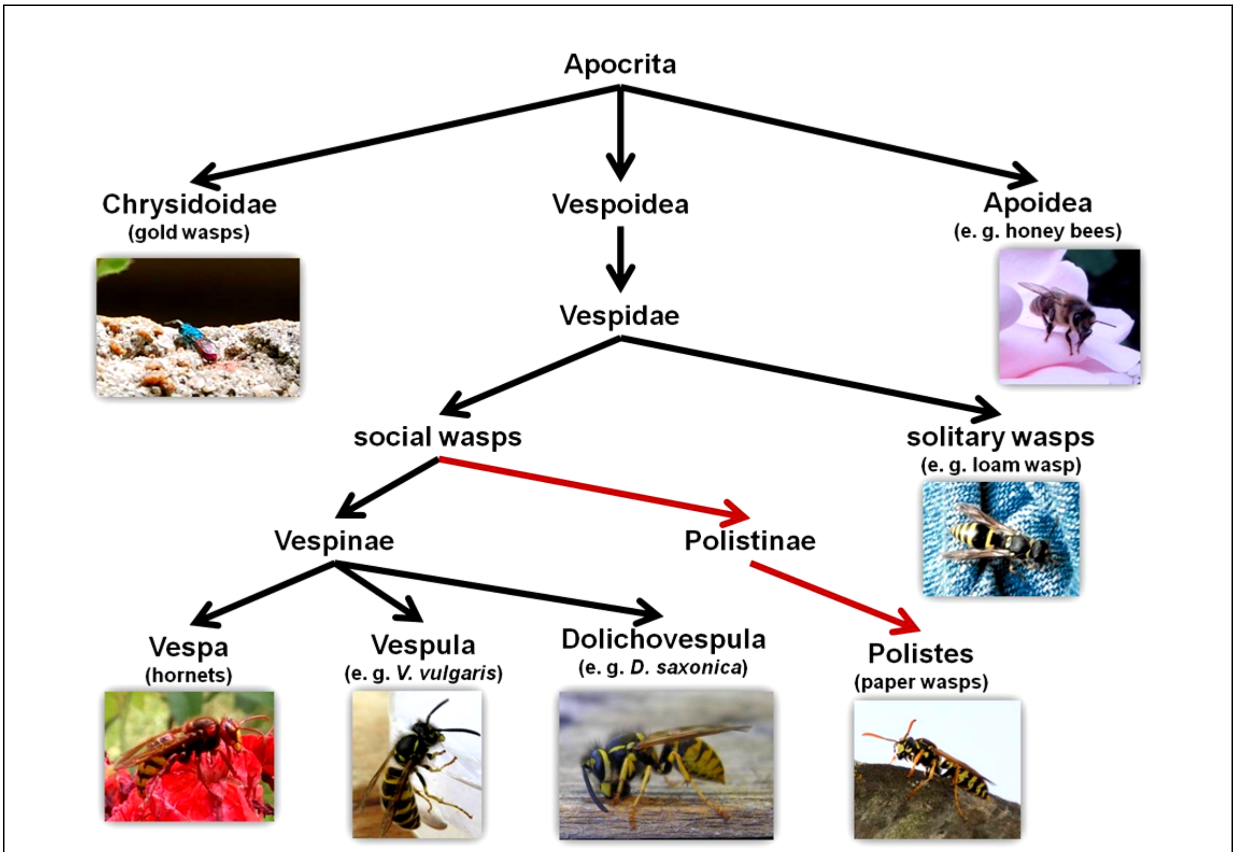


Fig. 1: Section of the insect family tree.
Polistes wasps are listed between the solitary and social living wasps. All pictures were taken by the author, with the exception of the photo of *V. vulgaris* (© and courtesy of D. Materna).

Furthermore, *Polistes* wasps express a lack of physiological caste differentiation and are therefore considered as primitively eusocial (Hunt 2006). Hence, they are an important model species for studies answering the evolution of eusociality and dominance hierarchies (West-Eberhard 2006). Particularly, the species *Polistes dominula* is often used for this kind of investigations, due to its wide distribution range and frequent occurrence. In Germany, five different species of *Polistes* are native, but *P. dominula* is the only species which is distributed throughout Germany and is constructing its nest in sheltered sites **(Table 1)**.

Table 1: Distribution and nesting sites of the different species of *Polistes* in Germany.

Scientific name	German name	Distribution	Nesting sites
Polistes dominula	Haus-Feldwespe	Throughout Germany	Warm, sunlit, sheltered sites (eternit panels, roof tiles, etc.)
Polistes nimpha	Heide Feldwespe	South and Central Germany, Mecklenburg Vorpommern & Brandenburg	Sunny habitats (Nests on plant stems or stones)
Polistes bischoffi	Zierliche Feldwespe	South Germany,	Sunny habitats (Nests on plant stems or stones)
Polistes biglumis	Berg Feldwespe	South Germany, especially in alpine regions	Sunny habitats (Nests on plant stems or stones)
Polistes atrimandibularis	Berg-Feldwespen-Kuckuckswespe	South Germany	Parasitic (social parasite of *P. biglumis)*

While numerous studies have been done on the behavioural ecology and the evolution of eusocial behaviour of these wasps (e. g. Field et al. 1998, Reeve et al. 2000, Tibbetts and Reeve 2003, Hunt 2006, Nonacs et al. 2006, Weiner et al. 2009, Weiner et al. 2013, Jandt et al. 2014), questions about the basic physiology remained unanswered so far (Weiner et al. 2010).

1.2 NESTING BEHAVIOUR OF *POLISTES DOMINULA*

Paper wasps are very successful with their primitively eusocial way of life. Particularly, *Polistes dominula* is a Palearctic species (Judd and Carpenter 1996), which is originally common in the Mediterranean region, North Africa countries and Middle Eastern countries, including parts of Russia and China (Carpenter 1996, Cervo et al. 2000). In the United States, *P. dominula* caused a stir because this species was introduced there in the late seventies (Hathaway 1981, 1982) and subsequently spread very fast throughout North America and Canada (Jacobson 1986, 1991a, 1991b, Menke 1993, Staines and Smith 1995, Judd and Carpenter 1996, Borkent and Cannings 2004, Hesler 2010). At the same time, this species was reported in Australia (Richards 1978) and in the early nineties in Chile (Willink and Chiappa 1993). In 2008, this paper wasp was detected in South Africa (Eardly et al. 2009) and almost every year there are new reports on the further spreading of this species worldwide.

In Europe, *P. dominula* is also expanding its habitats towards northern territories, however, these actions have attracted little attention so far. The first statement about the spread in Germany was reported by Blüthgen (1956). He mentioned that during the forties and fifties, *P. dominula* spread in North-West Germany without disclosing details about the exact sites. Until the eighties, it was assumed that *P. dominula* occur only “in the warmer parts of Germany” (Hathaway 1981). But 40 years after Blüthgen´s publication (1956), *P. dominula* was observed for the first time in Paderborn at a latitude of 51° (Lauterbach 1996) and only three years later in Hamburg at a latitude of 53° (Rathjen 1999). The first evidence of *P. dominula* in Belgium and the Netherlands was recorded in the mid-eighties (Simon Thomas 1984). In the Baltic countries, this species was first discovered in 1997 (Pekkarinen and Gustafsson 1999) and its further distribution in Lithuania has been described recently (Budrys et al. 2013). In order to understand why *P. dominula* is such a good invader in Central Europe, basic principles about nesting behavior of this species have to be examined in this distinct area. However, most published studies about *P. dominula* exist from the

Mediterranean region or the USA (reviewed by Ross and Matthews 1991, reviewed by Turillazzi and West-Eberhard 1996) which does not allow to draw conclusions on the behavior of this species in other regions.

As members of primitively eusocial wasps, foundress associations in *Polistes* species are quite normal (Reeve 1991; **Fig. 2)**. But there are great intra- and interspecific differences (reviewed by Jandt et al. 2014). In the case of *Polistes dominula*, the intraspecific variation does not only exist between ratios of multiple versus single founded colonies (Turillazzi et al. 1982, Zanette and Field 2011), but also between the relatedness of the co-foundresses (Queller et al. 2000, Zanette and Field 2008).

Fig. 2: Types of founding strategies of *P. dominula*.
The picture on the left shows a nest, founded by one queen (called: single founded), the picture on the right side illustrates a nest founded by three queens (called: multiple founded).

The number of foundresses per nest seems to depend very strongly on the investigated site, too. In a survey conducted in the northern parts of the United States, 1-6 foundresses per nest were found (Tibbetts and Reeve 2003). In another study in Spain, up to ten foundresses per nest were quite normal (Zanette and Field 2009). Reeve (1991) was able to show that the frequency of multiple-foundress associations is positively correlated with the nest failure of single-foundress nests in all investigated *Polistes* species. Tibbetts and Reeve (2003) detected a survival rate of 68% for single founded colonies at the study sites in the USA. However, multiple founded nests survived with a rate of 94% even better at the same sites. A second key advantage of multiple founding is the ability to build larger nests (Pickett and Wenzel 2000, Tibbetts and Reeve 2003) which opens up the possibility to raise more queens and drones (Pickett and Wenzel 2000). Therefore, the strategy of multiple founding seems to be preferable and almost a guarantor for survival.

1.3 THERMOREGULATION OF INDIVIDUAL PAPER WASPS

Our knowledge of thermoregulatory mechanisms of *Polistes dominula* wasps is very limited. However, thermoregulation is very important in some highly social insects and well investigated in this species. Scientists distinguish between two different types of thermoregulation: First, the thermoregulation at the level of the individual insect (e. g. Heinrich 1984, reviewed by Heinrich 1993, Coelho and Ross 1996, Kovac and Stabentheiner 1999, Coelho 2001) and second, the control of nest temperatures (e. g. Steiner 1930, reviewed by Heinrich 1993, Hozumi and Yamane 2001, Klingner et al. 2005, Klingner et al. 2006, reviewed by Jones and Oldroyd 2007).

It is known that large endothermic insects must achieve a minimum threshold temperature during foraging flights (e. g. Heinrich 1979, Coelho and Ross 1996, Kovac and Schmaranzer 1996, Woods 2005). Accordingly, many large insects increase their thorax temperature shortly before flight (reviewed by Heinrich 1993, Coelho 2001). The elaborate pre-flight warm-up behaviour, which was first demonstrated in moths, is known to be very important in many social insects such as honey bees or bumble bees (reviewed by Heinrich 1993). Honey bees need a thorax temperature above 30°C for flight activities (reviewed by Woods et al. 2005). For flying at lower ambient temperatures, honey bees usually warm-up their wing muscles before (reviewed by Heinrich 1993). If an insect is able to heat up its flight muscles before it starts flying, it has the advantage to be able to fly at cooler ambient air temperatures **(Fig. 3:** N. Höcherl, personal observation).

As already proven for bumble bees (Heinrich, 2001), also some wasp species forage nectar at remarkably low outside temperatures. Heinrich (1993), observed *Vespula* queens foraging on the tundra (northern Alaska) at air temperatures near 5°C. He noticed that the thoracic temperature of the observed wasps were nearly 30°C which is slightly above the minimum for flight activity.

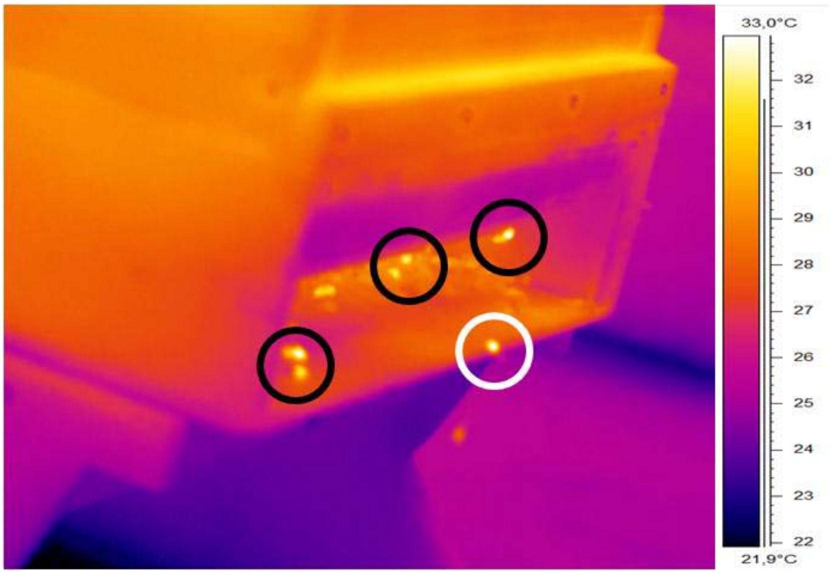


Fig. 3: Thermographic image of a bee hive entrance.
The white circle marks a home coming bee, whereas the black circles indicate bees showing the pre-flight warm-up behaviour. Temperature scales are on the right side.

In a field study, Kovac et al. (2009) compared the thermoregulatory behaviours of water foraging wasps (*Polistes dominula* and *Vespula vulgaris*). The authors were capable of proving that at moderate ambient temperatures *Polistes* wasps show a weaker endothermic activity than *Vespula* wasps, whereas at higher temperatures *Polistes* wasps exhibit always an ectothermic behaviour, i. e., a passive adaption of all body regions to the ambient temperatures.

Weiner et al. (2010) measured thorax, head and abdomen temperatures of inactive *P. dominula* workers as they warmed up after transfer from 8 to 25°C ambient temperatures. Body temperatures were measured by inserting a microprobe thermocouple, connected to a thermometer. They found that thorax temperatures of non-flying wasps increased more rapidly than that of dead wasps and that some living wasps reacted more than 2°C above ambient temperature indicating endothermy. Moreover, they observed that flying *P. dominula* wasps stabilized their thorax temperature in response to changes in air temperature and that *Polistes* queens maintained slightly elevated temperatures after removal from hibernation clusters.

1.4 THERMOREGULATION OF *POLISTES DOMINULA* NESTS

As mentioned in the previous paragraph (1.3), scientists distinguish between two different main types of thermoregulation: The control of temperature at the level of the individual insect and the regulation of nest temperatures. Nests of social insects can be considered as factories for the production of offspring (Heinrich 2001). Since larvae of social insects often react very sensitively and sometimes with abnormalities in development to elevated or extreme variations in temperature, the regulation of nest temperature is of utmost importance (e. g. Himmer 1927, 1936, Heinrich 2001, Tautz et al. 2003, reviewed by Jones and Oldroyd 2007). Therefore, many social insect species are able to regulate the temperature within their nests (Jones and Oldroyd 2007). Jones and Oldroyd (2007) have divided the mechanisms of nest thermoregulation in two categories: active and passive thermoregulation. The active mechanisms include all types of thermoregulation which can be attributed to the behaviour of individuals to modify the nest temperature **(Table 2)**.

Table 2: Outline of active thermoregulatory mechanisms used in different social insect groups.

Insect group	**Active thermoregulation**			
	Response to low temperatures		**Response to high temperatures**	
	Clustering	**Heating**	**Fanning**	**Water evaporation**
Ants	S	N	N	N
Honey bees	A	A	A	A
Stingless bees	S?	?	S	N
Bumble bees	A	A	A	N
Polistinae wasps	**?**	**N?**	**A**	**A**
Vespinae wasps	M	M	A	S
Termites	S	N	N	N

S = Some species; M = Most species; A = All species; N = no Species (modified from Jones & Oldroyd 2007)

One of the two behavioural responses to low temperatures is the clustering of individuals. It is known from most social bee and some Vespine wasp species that workers are able to generate metabolic heat while clustering on the surface of brood cells (Ishay 1973, Fahrenholz et al. 1989). This behaviour is – to some extent – also perceptible in primitively eusocial insect species such as *Apoica flavissima* (Yamane et al. 2009). The other possibility to respond to low temperatures is the active heating i. e., direct incubation of the brood. This behaviour is known from all honey bee and bumble bee and some hornet species (Ishay 1973, reviewed by Heinrich 1993, Bujok et al. 2002).

The responses to high temperatures are wing fanning or evaporative cooling. It does not have to be particularly emphasized that fanning only occurs in species with wings. But all other social insect species display this cooling behaviour. Fanning is defined as the behaviour of alate workers standing in a stationary position while fanning their wings to remove warm air from the nest **(Fig. 4)**. Evaporative cooling, however, means that water-collecting workers apply drops of water on the surface of the brood comb (Jones and Oldroyd 2007).

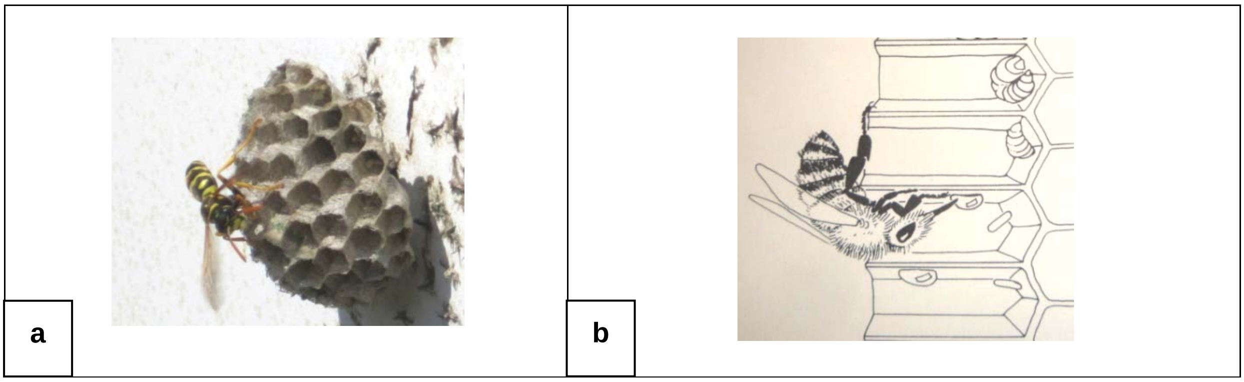


Fig. 4: Cooling behaviour of social insects.
(a) A fanning *P. dominula* wasp; (b) A schematic representation of the water application by a bee to a brood cell (from Seeley 1997).

Passive thermoregulation of nests includes all mechanisms like site selection or special structures of nests which help to optimize the stabilization of the internal temperatures **(Table 3)**.

Table 3: Outline of passive thermoregulatory mechanisms used in different social insect groups.

Insect group	**Passive thermoregulation**		
	Nest orientation	**Nest architecture**	**Nest site selection**
Ants	S	S	M
Honey bees	S	S	A
Stingless bees	?	M	A
Bumble bees	S	A	A
Polistinae wasps	**?**	**S**	**A**
Vespinae wasps	?	A?	A
Termites	S	S	S

S = Some species; M = Most species; A = All species; N = No species (modified from Jones & Oldroyd 2007)

For example, with the help of nest orientation some social insects influence the amount of solar radiation absorbed by a nest. They turn the surface of the nest away from or towards the sun depending on whether they live in hot or cold areas of the world (Jones and Oldroyd 2007). However, in wasp species this kind of passive thermoregulation has not yet been documented. Within social insects, the nests vary tremendously in structure and material (wax, paper, soil, etc.). But all the different designs help to stabilize the temperature inside the nest (Jones and Oldroyd 2007). Additionally, wasp species build completely different nest structures **(Fig. 5)** and hence utilize different types of passive thermoregulation.

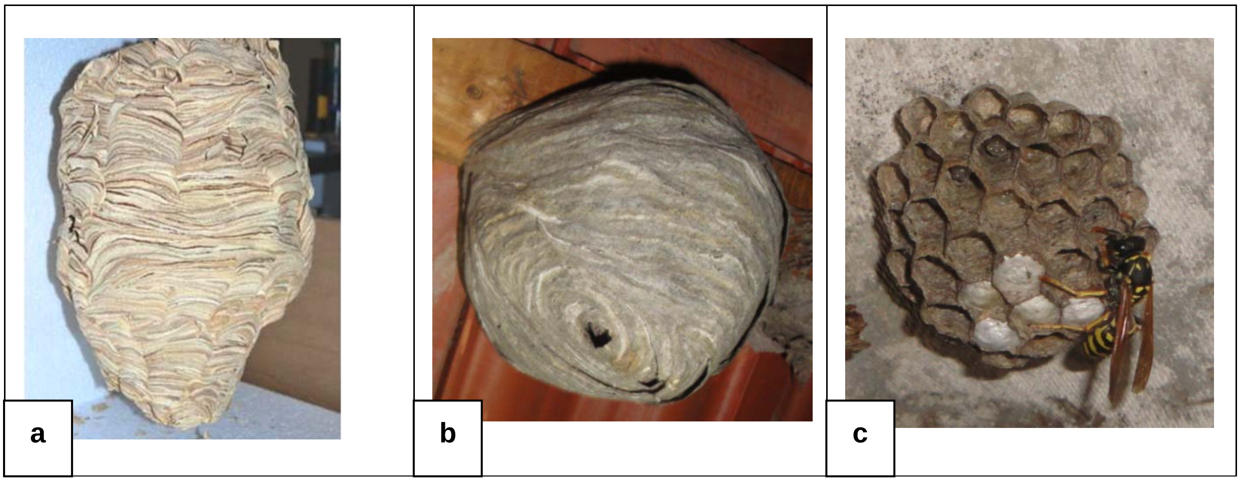


Fig. 5: Different structures of wasp nests.
(a) A nest of the hornet *(Vespa crabro)* with an envelope and visible air pockets; ca. 55 cm high; (b) A nest of the saxon wasp *(Dolichovespula saxonica)* with a multilayer envelope but no air pockets; diameter ca. 25 cm; (c) A nest of the paper wasp *Polistes dominula*, without envelope; diameter ca. 6 cm.

Most wasps rely on an envelope around the nest, but others use nest site selection for stabilization of the nest temperature. For example, the German wasp *(Vespula germanica)* is nesting in the ground, which helps to maintain perfect conditions for its brood. However, wasps of the genus *Polistes* build exposed combs, without any cover and protection. Due to this fact, the thermoregulation of these nests depends predominantly on the environmental conditions at warm sheltered nesting sites (Jeanne and Morgan 1992).

Finally, it should be pointed out that not a single mechanism is sufficient for maintaining a stable temperature inside the nest. Usually, several mechanisms – both active and passive – are utilized at the same time.

1.5 The aims of this work

As summarized, all comprehensive studies about the nesting behaviour of *Polistes dominula* were conducted either in South Europe or in the United States of America and nearly nothing was known about *P. dominula* in Central Europe – apart from the distribution of this species. Moreover, data spanning a period of time longer than one colony cycle are scarce. Therefore, the basic principles about nesting of *P. dominula* wasps in Germany were investigated for three consecutive years, at intervals of one week, always at the same six nesting sites spanning the begin of the nest founding up to the end of the season. Foundress associations, number of foundresses per nest, survival rate, colony cycle length and nest sizes were estimated to reveal specific features in founding strategies that would eventually explain the successful spreading of *Polistes dominula* in Central Europe.

Individual *P. dominula* wasps have been demonstrated to control their temperature during flight, but it was unknown so far whether they are able to respond to specific hazards on the nest by elevated thorax temperatures. Therefore, the thorax temperature of adult paper wasps was measured under non-flying situations and in the direct environment of their nest. The thorax temperature of adult *P. dominula* wasps was monitored with a thermographic camera while they were exposed to various stressors on their nests. Real threats were simulated, like a fire nearby, by the application of smoke, or a predator attack by exposing the animals to carbon dioxide (CO_2). Additionally, the thorax temperature of resting wasps and of queens during dominance battles was investigated. This was done to elucidate potential functions of the thermoregulation on the level of the individual insect.

Many different types of nest thermoregulation are known. However, the thermoregulatory mechanisms in a *Polistes dominula* nest had been investigated only once, in the late 20s (Steiner 1930). This very impressive work revealed that the adult wasps showed fanning and cooling by water evaporation. By using temperature data loggers, the ambient temperature of nests at different and identical sites was measured during three consecutive years. Furthermore, active and non-active nests were monitored simultaneously and different nest sizes were investigated with a thermographic camera. Using these “novel” methods, it was tried to find more and much clearer results about the thermoregulatory mechanisms in *P. dominula* nests.

2. MATERIALS

2.1 EQUIPMENT

Air pump B´win FLP 1000	B´win company
CO_2 Reusable Cylinder	DENNERLE
EL-USB-2-LCD RH/TEMP Data Logger	LASCAR electronics
Endoscope camera (flexible)	SOMIKON ®
Paint marker Edding 750	Edding
PC Thermofox (Data logger)	Scanntronik; Mugrauer GmbH, Zomeding-Pöring
PC Multisensor Temperatur (for Thermofox)	Scanntronik; Mugrauer GmbH, Zomeding-Pöring
Pressure Reducer (for CO_2 gas bottle)	DENNERLE
Smoker (for Beekeepers)	
Sony Cybershot; DSC-W270	SONY®
Sony HANDYCAM; DCR-SR190E	SONY®
Thermo camera FLIR SC 660	FLIR Systems™
Tripod	CULLMANN

2.2 SPECIAL SOFTWARE

Adobe® Illustrator® CS4	Software for image editing, Adobe®
EasyLog USB	Software for EL-USB-2-LCD; LASCAR electronics
SoftFOX 2.73	Software for Thermofox; Scanntronik; Mugrauer GmbH, Zomeding-Pöring
STATISTICA Version 10.0	Statistic-Software; STATSOFT
ThermaCAM™Researcher	Software for Thermocamera; FLIR Systems™

3. METHODS

3.1 GENERAL REMARKS

3.1.1 Selection of suitable nesting sites

In Germany, *P. dominula* colonies are found almost exclusively in man-made structures (Blüthgen 1956, Bleidorn et al. 2000). Due to this fact, different nesting sites were chosen. During the winter months of 2011/2012, ten different sites in and in the vicinity of Würzburg (within a 26 km radius) were discovered. Four locations were houses with non-isolated roofs. Three sites were barns with a roof built of corrugated eternit panels. Two other sites were wooden boxes (58 x 52 x 31 cm) standing in a garden. The last site was made up of metal tubes (5.5 cm diameter) at a vineyard. The study on newly founded nests was conducted at six of the ten sites in and near Würzburg (within a 5.5 km radius) in spring and summer of 2012, 2013 and 2014.

3.1.2 Selection of suitable *Polistes dominula* nests for thermographic studies

Not all of the discovered newly founded nests were suitable for thermographic recordings. All nests – without any exception – were found within barns, wooden boxes, metal tubes and other constructions related to human habitats. Openly hanging nests of *P. dominula* without roof beams or other structures directly under the nests were indispensable to get satisfactory images. At the end, eleven nests at four different sites (barns) were suitable for thermographic recordings during the study periods. All eleven nests were used for investigating the thermoregulatory mechanisms of the nests and for the first part of thermoregulatory mechanisms of individual *P. dominula* wasps. For the last-mentioned study (testing the effect of stressors), a nest was observed after emergence of at least five workers. This was usually the case at the end of June. For the second study about thermal behaviour of individuals (dominance battles), nests were investigated only during the founding phase. Five of the eleven nests were founded by two or more queens within the three spring seasons. The founding phase was defined as the time between the first cells were built and the first adult workers had been emerged.

3.2 INVESTIGATION OF THE NESTING BEHAVIOUR OF *P. DOMINULA*

3.2.1 Screening of abandoned *Polistes dominula* nests

During the winter months of all three study years (2011-2014), all ten sites in and near Würzburg were inspected and all nests were taken down and collected. After collecting them, the number of cells was counted and the nests were stored for other experiments in wooden boxes **(Fig. 6).**

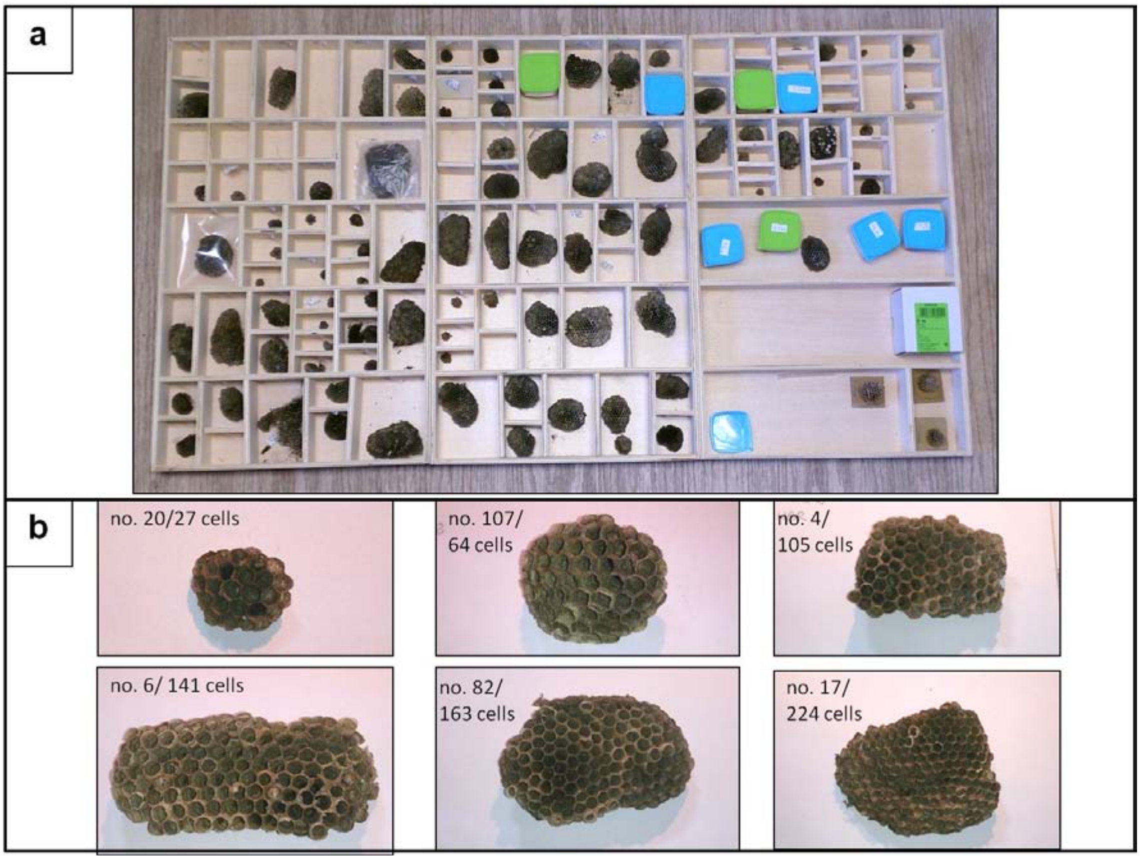


Fig. 6: The preservation of nests.
(a) The preservation of *Polistes* nests in three wooden boxes. (b) Selected nests of different sizes. Nest no. 20 consists of 27 cells (site 3); nest no. 107 is made up of 64 cells (site 6); nest no. 4 consists of 105 cells (site 2); nest no. 6 is composed of 141 cells; nest no. 82 is made up of 163 cells (site 8) and nest no. 17 of 224 cells (site 10).

3.2.2 Observation of nesting season and nest sizes of newly founded *Polistes dominula* nests

In spring, after the first cells were visible (= start of the nesting season), colonies of *P. dominula* were studied in their natural environment. The nests were inspected once per week during the whole nesting seasons (March – September) of the three years by counting cells, adult animals and brood cells containing pupae. The season was over, when new wasps were no longer hatching (= end of nesting season). In order to be able to document the nests within the metal tubes, a dentist-mirror and a flexible endoscope (SOMIKON®) was used.

3.2.3 Recording of foundress associations

The number of foundresses was recorded after detection of the respective nest and verified during the following weeks until the first workers emerged (= end of founding period). To ensure that all queens were present during the observation times, censuses were performed early in the morning.

3.2.4 Recording of survival rate and productivity

At the end of the founding period, the intact nests were counted and the survival compared with the number of foundresses. A nest was classified as "failed" if all foundresses disappeared during the founding period and no adult had emerged from this nest (Tibbetts and Reeve 2003). To determine the productivity of the nests depending on either single- or multiple-foundress colonies, the number of cells of each nest was documented at the end of the founding period and at the end of the season (Tibbetts and Reeve 2003). As another criterion for productivity, the mean number of pupae of each nest (single- and multiple-foundress) at the end of the founding period was recorded.

3.3 INVESTIGATION OF THERMOREGULATORY MECHANISMS OF INDIVIDUAL *POLISTES DOMINULA* WASPS

3.3.1 Challenge of *Polistes dominula* individuals with stressors

In a first step, different stressors were examined before starting the trial. Hence, the most promising and quantifiable stressors were smoke and carbon dioxide (hereafter called: CO_2).

A customary bee smoker was used for stressing *P. dominula* wasps with smoke (generated from poplar wood), simulating a fire near the nest. For testing the animals with CO_2, a gas bottle was used. This stressor simulated the attack of a larger predator, because CO_2 is highly concentrated in the exhaled air of animals. To ensure that not the air blast of the smoker or the gas bottle itself caused the reaction of the wasps, an air pump was used as control **(Fig. 7)**.

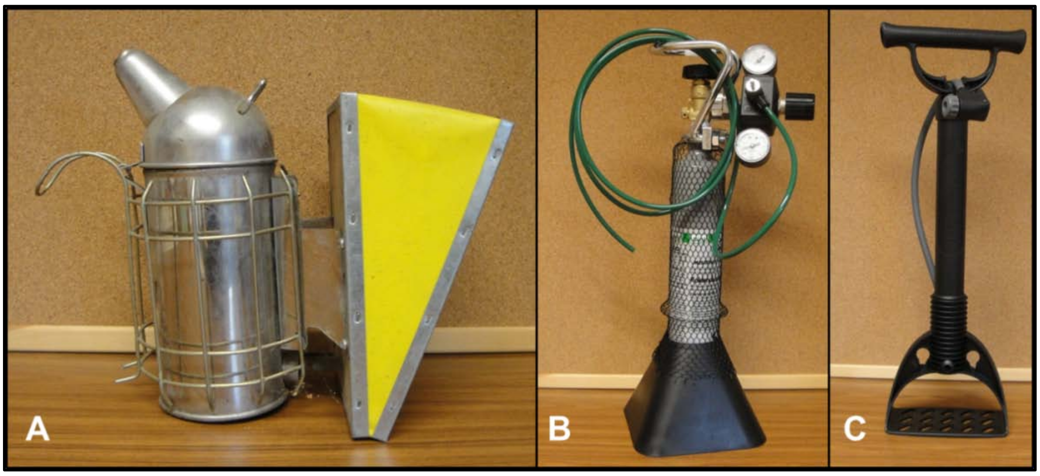


Fig. 7: Instruments used for stressing *P. dominula* individuals.
The bee smoker (A); a CO_2 gas bottle (B) and an air pump (C).

The recording of the possible reaction of adult *P. dominula* wasps was accomplished by a thermographic camera. The wasps were filmed without disturbing them. The camera was calibrated periodically by the manufacturer. The monitoring of the ambient temperature was done by temperature data loggers (Thermofox; Scanntronik). Additional reference measurements were not necessary according to the manufacturer of the thermographic camera (FLIR). The camera was adjusted to take a picture every ten seconds. In order to get data of the animal´s thorax temperature before being threatened, the nest was observed for at least four minutes before using the respective stressor. The stressor was then applied for three seconds. It should be pointed out that smoke and CO_2 concentration was maximal during the three second application period and that it dropped quickly thereafter. To make sure that movements in the neighbourhood of the nest did not trigger the reaction of the animals, the stressor was administered in a distance of at least 20 cm from the nest. The reaction of the adult wasps was recorded for at least five minutes after the stressor was applied. Every suitable nest was filmed at least for one time, but mostly for more than two times. Recordings were discarded when less than four animals were visible on the nest for a longer time than 20 seconds. Additionally, recordings were cast aside if wasps started to fan (for a longer period than 5 s) after the stressor was applied. This behaviour also leads to an increased thorax temperature (N. Höcherl, personal observation). The experimental series were carried out 20 times with smoke and 20 times with CO_2. The reactions to the air blast of a pump were investigated 12 times. Additionally, the thorax temperatures of at least four resting wasps were monitored for 12 times to investigate possible elevated thorax temperatures during resting phases of the wasps. To ensure that the wasps did not leave the nest during a trial, the experiments were only conducted during the night, or on cold (max. temperature under 20°C) or rainy days.

3.3.2 Observation of dominance battles

To investigate the dominance battles, multiple founded nests of *P. dominula* were observed with the infrared camera during the whole day, because I was not able to prognosticate when such fights will happen. A distinction between dominant and subordinate queens was possible by a longer observation of the nest. The dominant queen was defined as the animal that remained on the nest during the day, was fed by the other queens and was laying the eggs. This animal was marked with a small coloured dot on the abdomen. Additionally, the dominant queens were resting directly on the anterior side of the comb (Pardi 1996), whereas the subordinate queens often rested on the edge of the comb or in the small gap between nest and roof. Most of the battles took place at night, during which time the wasps usually reside motionless on the comb. A dominance battle between a subordinate and a dominant queen was considered as such, if they changed continuously their position, circled each other for more than 30 seconds and had direct contact again and again. The settings of the camera were the same as described above. Twelve dominance battles were investigated. The whole fights were analysed regardless of how long they lasted.

3.4 INVESTIGATION OF THERMOREGULATORY MECHANISMS OF *POLISTES DOMINULA* NESTS

3.4.1 Measurement of the influence of adult paper wasps on the nest temperature

3.4.1.1 Recording of the nest temperature with the help of thermography

To investigate the nest temperature the thermographic camera was used, too. In a first step, single shots at different times of the day were taken to get a first impression of nest and roof temperatures. The camera was adjusted like described above (see 3.3.1). 24h series were then taken to get an overview about the nest temperatures during the whole day. During the study seasons in summer 2012 and 2013, 16 shots with adult wasps and 13 without adult wasps on the nest were taken. The observation with the camera was performed every two hours between 8.00 am until the next day 8.00 am (24 h) for ten minutes per data point. After these shots were analysed, it became apparent that no important thermoregulation takes place during the night (8.00 pm – 8.00 am).

With a modified experimental setup, the trial was performed again in 2014. Five nests (no. 46, 47, 53, 62 and 63; see **Table S1**) were selected for this study. Old abandoned nests (described in 3.2.1; see **Table S2**) were placed in the direct vicinity of the newly established nests. The old nests had the same size (±10 cells) as the newly founded nests at the time of data collection **(Fig. 8).**

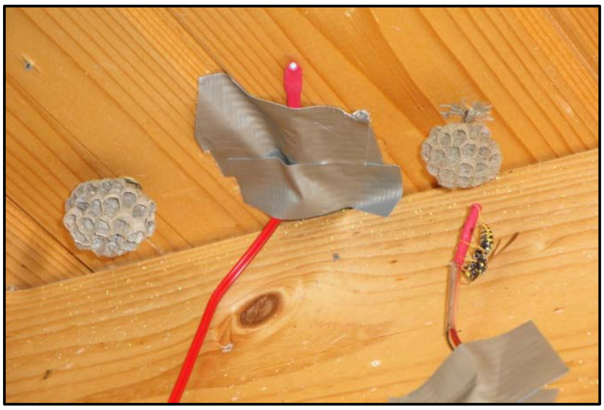


Fig. 8: Experimental setup for the recording of the nest temperature with the help of thermography.
On the right side, the newly founded nest no. 63 (site 3) is seen; on the left side, an old abandoned nest of similar size has been glued to the roof. The red sensors are temperature sensors for recording the ambient temperature during the whole experiment. The old abandoned nests were changed accordingly to the growth of the newly founded nest.

This was a guarantor for getting data about the influence of only the adult paper wasps on nest temperature. The observation with the infrared camera was performed every two hours between 8.00 am to 8.00 pm (12 h) for ten minutes per data point. The recordings were made at different maximum outside temperatures, measured with the Beestations own weather station (Campbell scientific cr 1000; sensor: cs215). The nests were observed in a temperature range between 15 and 35°C (± 1) maximum temperature during the whole day **(Table 4).**

Table 4: Overview about the number of observed nests at the different outside temperatures.

Maximum temperature outside [°C]	Number of observed pairs of abandoned and newly founded nests
15	5
20	5
25	3 a)
30	5
35	3 a)

a) Two nests were destroyed by birds during the current study

3.4.1.2 Recording of the efficiency of cooling the nests by fanning and water evaporation

During the studies in 2014 on 5 different newly founded nests with the infrared camera (described under 3.4.1.1) the behaviour of the adult wasps was recorded, too. Every two hours (between 8.00 am and 8.00 pm) the nests were observed for ten minutes. It was documented whether the adult *P. dominula* wasps showed a fanning behaviour or if home coming wasps brought water for cooling the nest dependent on the temperature of the day. Wasps, which brought water for cooling, were detected with the help of the infrared camera. Cooled cells appeared in dark colours after the wasp had applied the water to the cell walls.

In addition, thermographic images of cooling events were analysed. If a wasp was sitting on the nest and showed a fanning behaviour, the temperature of the cells in the direct vicinity of this wasp was analysed. This was done to record whether and to which extent this behaviour had a cooling effect. Only four cases of fanning behaviour on the nest could be documented by thermography during the three years of study (2012 – 2014). Furthermore, the efficiency of cooling single cells by water evaporation was analysed. The temperature of single cells (n = 42) was measured after the home coming water collecting paper wasp had applied the water to the cell walls. The consecutive thermographic images were analysed until they nearly reached the outlet temperature. This was done in order to record to what extent this behaviour had a cooling effect.

3.4.2 Measurement of the influence of the nest size on the nest temperature

To investigate the influence of the nest size on nest temperature, old abandoned nests (from 3.2.1) were used. It became apparent that nests between 31 and 150 cells were the most abandoned nests in this study area. Cohorts of 30 cells were formed (31-60; 61-90; 91-120; 121-150 cells). Three absolutely intact nests of each cohort (n = 12) were selected. The nests were glued on a piece of paper (80g/cm^2) and attached on a natural nesting site of *P. dominula* **(Fig. 9)**.

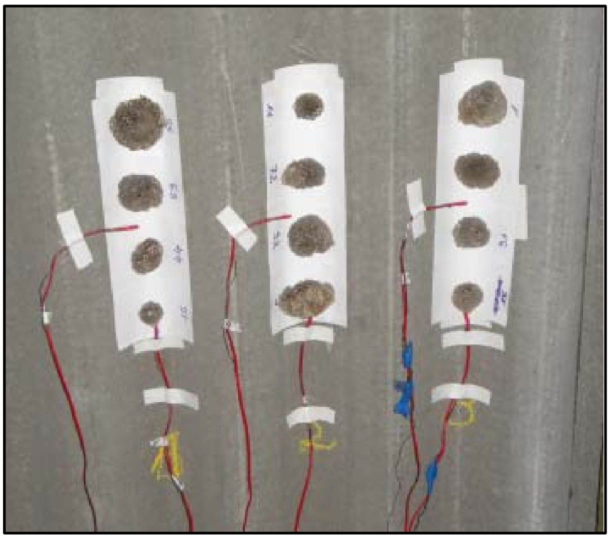


Fig. 9: Experimental setup of old abandoned nests of the four different size-cohorts for investigating the influence of nest size on nest temperature.
Twelve nests (three of each size cohort) were fastened on a natural *P. dominula* site. This site was a barn with a roof consisting of corrugated eternit panels. Because of the pitched roof and as a consequence a possible difference in temperature development, the nests were hung up in different orders.

This setup was equipped with temperature data loggers and in addition investigated with the help of the thermographic camera. Recordings were made every two hours for at least 10 minutes between 24 h of observation (8.00 am to the next day 8.00 am). The nests were observed in a temperature range between 10°C and 35°C (in 5°C-steps) maximum temperature during the whole day.

3.4.3 Measurement of the influence of the site on the nest temperature

For investigating the influence of the site on the nest temperature, 21 nests were equipped with temperature data loggers during the three years of this study. Partly it was possible to equip more than one nest at one site in the same year with a data logger. The loggers were adjusted to record the temperature every ten minutes **(Fig. 10).** The loggers were readout once per week.

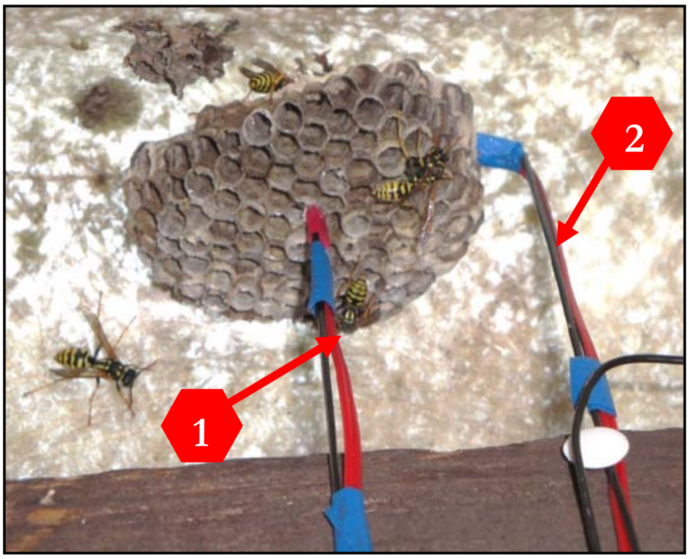


Fig. 10: Experimental setup for investigating the influence of the nest site on the nest temperature.
Two temperature sensors were affixed to one nest. The first sensor measured the temperature of the direct ambience of the nest (1) and the second sensor measured the temperature between the nest and the roof (2).

In addition it was partly possible to take thermographic shots at the same time frame (within 30 min) at different times (as described under 3.4.1.1). This gives data about the influence of the microclimate at one nesting site compared to another, too.

3.5 DATA EVALUATION AND STATISTICAL ANALYSES

3.5.1 General remarks

The Software STATISTICA Version 10.0 (STATSOFT) was used for statistical analysis. The Software ThermaCAM™Researcher (FLIR Systems™) was used to analyse the thermographic pictures. The surface temperatures of the thorax were calculated with an infrared emissivity value of 0.97, which was determined for honeybee cuticle (Stabentheiner and Schmaranzer 1987, Schmaranzer and Stabentheiner 1988) and which is meanwhile established for investigating the surface temperature of wasps, too (Kovac et al. 2009). Graphical representation was done using Microsoft Excel 2010 and Adobe® Illustrator® CS4.

3.5.2 Analysis of the nesting behaviour of *Polistes dominula*

The survival rate was statistically checked by the X^2-sixfold-test. Productivity depending on multiple or single founding was tested by using the Mann-Whitney U-Test.

3.5.3 Analysis of the thermoregulation of individual *Polistes dominula* wasps

The hottest point of the thoraces of the involved wasps was examined. The thorax temperatures of at least four animals per picture were pooled for the effect of smoke, CO_2 and air. Mean and standard deviation of every picture was calculated. Six pictures (60 s) of a trial – before the stressor was applied – were analysed. After the stressor was applied, at least three minutes (18 pictures) were analysed. Additionally, the temperatures of at least four resting wasps were measured, the data points were pooled and mean values calculated. All calculated mean values were then subtracted from the first determined mean value of the thorax temperature to get comparable data, completely independent of ambient temperatures.

For investigating the dominance battles, the analysis started 4 pictures (40 s) before the fight began and it ended if the queens remain quiet again on their nests and achieved nearly the thorax temperatures as before the fight. We examine the hottest point of the thoraces of the involved queens. As described above, all documented values were subtracted from the first determined thorax temperature, to get comparable data sets.

Differences in the reaction of the wasps between the different stressors (smoke, CO_2) and between stressors and the negative control (air) were tested by using the Mann-Whitney-U-Test. Similarly, differences in the thoracic temperature of dominant and subordinate queens during a dominance battle was analysed by using a Mann-Whitney-U-Test.

3.5.4 Analysis of the thermoregulation of *Polistes dominula* nest

For investigating the effect of adult animals on the nest temperature, four positions were fixed for the nest temperature of the newly founded and also four positions within the old abandoned nest. In addition, four positions were fixed for recording the roof temperature. Eleven consecutive thermographic pictures per data point were analysed. The data of the nest temperatures and the roof temperature were pooled and the mean was calculated. To test the effect of the adult animals on the nest temperature with increasing outside temperature, a two-way analysis of variance (two-way ANOVA) was implemented.

To study the dependence of the behaviour of the adult paper wasps (construction material or food collection; fanning or water collection for cooling the nest) on increasing outside temperatures was tested with a Chi-square-Test. For every nest and time point a mean per quantified behaviour was calculated.

For analysing the effect of nest size on nest temperature, four points within every nest was fixed (12 nests = 48 sample points). Additionally, eight monitoring points were fixed for the roof temperature. Eleven consecutive thermographic pictures per data point were analysed. For every nest size cohort, a mean per day was calculated. To test the possible effect of nest size on nest temperature depending on increasing outside temperature, a two-way ANOVA was performed.

4. RESULTS

4.1 THE NESTING BEHAVIOUR OF POLISTES DOMINULA

4.1.1 Screening of abandoned nests

During the winter months of the four years 2011 – 2014, abandoned *P. dominula* nests were collected (n = 147) from ten different sites in the vicinity of Würzburg (within a 26 km radius) **(Fig. 11)**.

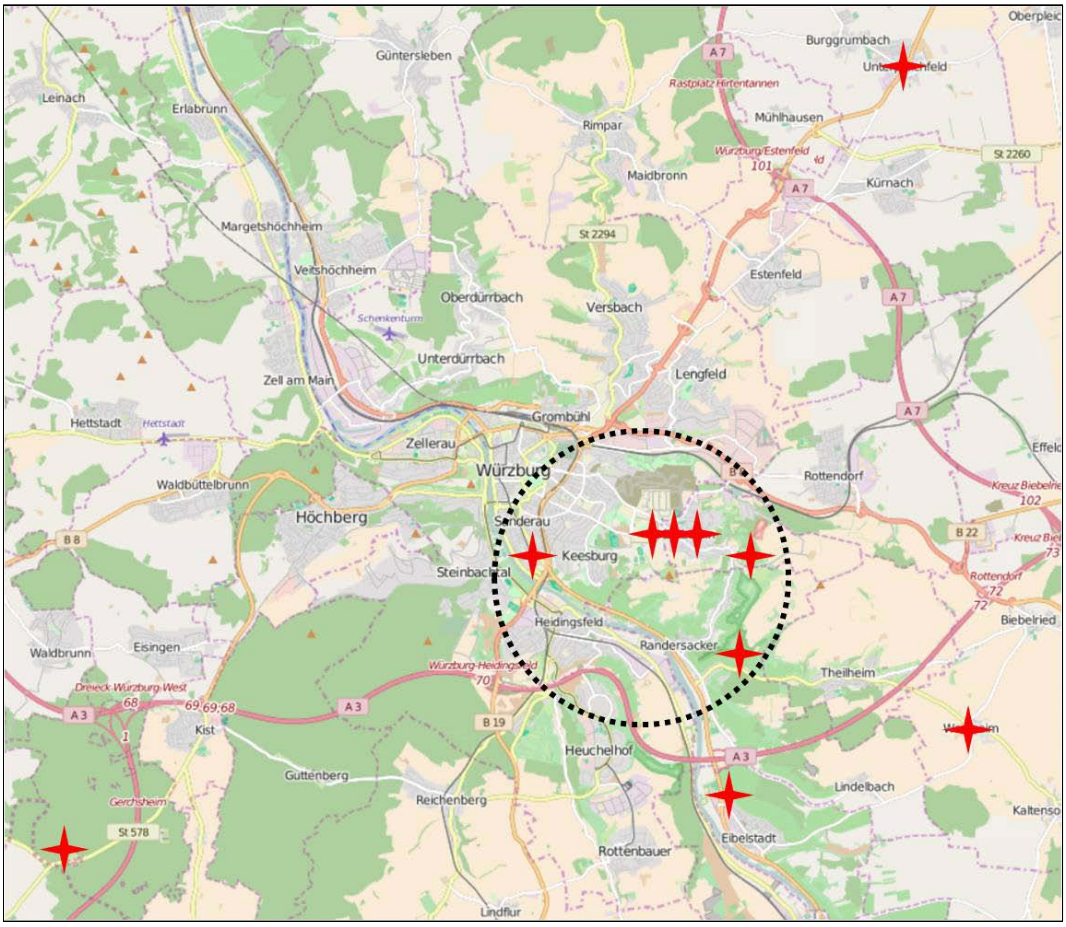


Fig. 11: Map of the nesting sites of *P. dominula* in a radius of about 26 km in and in the vicinity of Würzburg, Germany.
Map of study sites (©OpenStreetMap-Mitwirkende: www.openstreetmap.org). Red stars represent the ten sites, where abandoned nests of *P. dominula* (n = 147) were collected during the winter seasons of three years (radius = 26 km). The dotted black circle shows the area with the six study sites of newly founded nests of the summer seasons 2012, 2013 and 2014 (radius = 5.5 km) where the observations of 63 nests took place.

Four of the sites were houses with non-isolated roofs. Within these houses, a total number (n) of 32 nests was found. Three sites were barns with a roof built of corrugated eternit panels (n = 56). Two other sites were wooden boxes (58 x 52 x 31 cm) standing in a garden (n = 41). The last site was made up of metal tubes (5.5 cm diameter) at a vineyard (n = 18). In order to get a first indication of the average size of nests built by *P. dominula* in Germany, I counted the cells of abandoned nests from ten different sites in the vicinity of Würzburg. Most (67.4%, n = 99) of all collected nests (n = 147) consisted of sizes between 31 and 150 cells **(Fig. 12)**. Only 9.5% (n = 14) were composed of 151 cells and more. The remaining collected nests (23.1%, n = 34) were smaller than 31 cells. The mean size of abandoned nests (n=147) built by *P. dominula* in the studied area was on average 77.6 (± 55.4) cells.

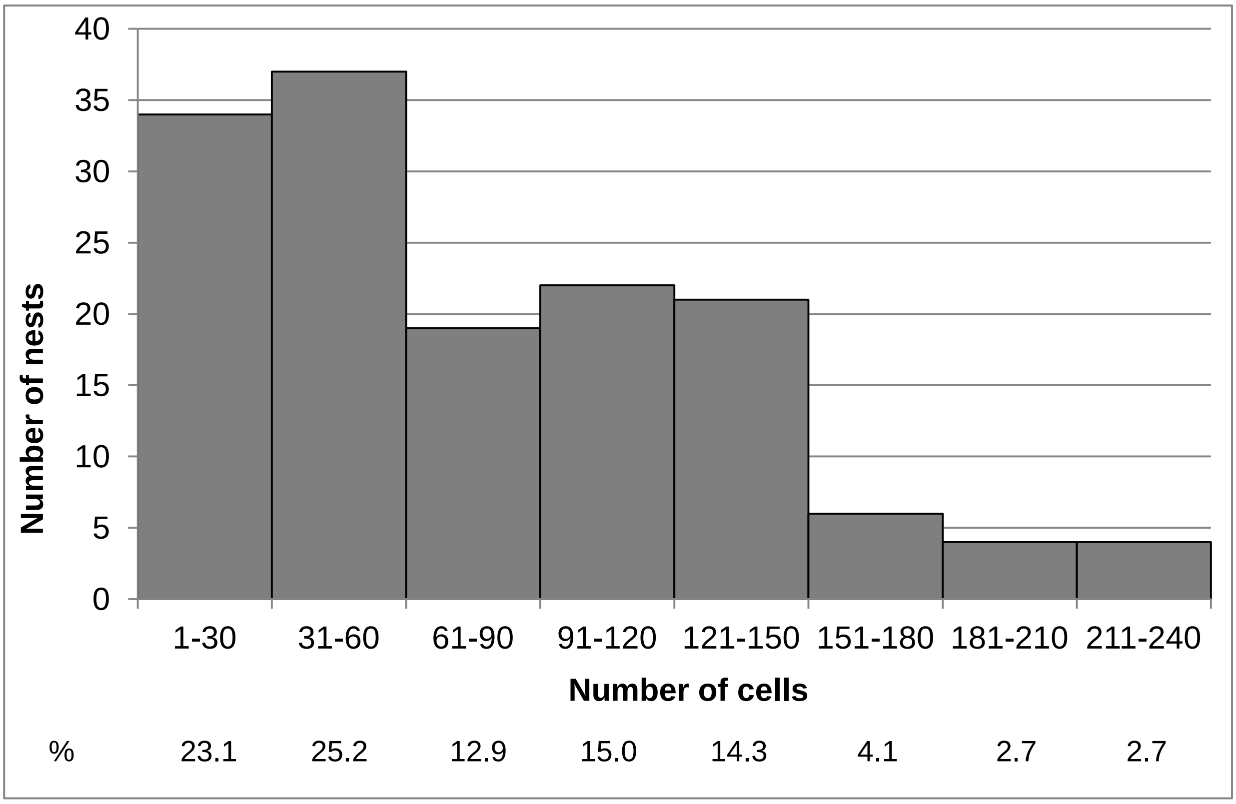
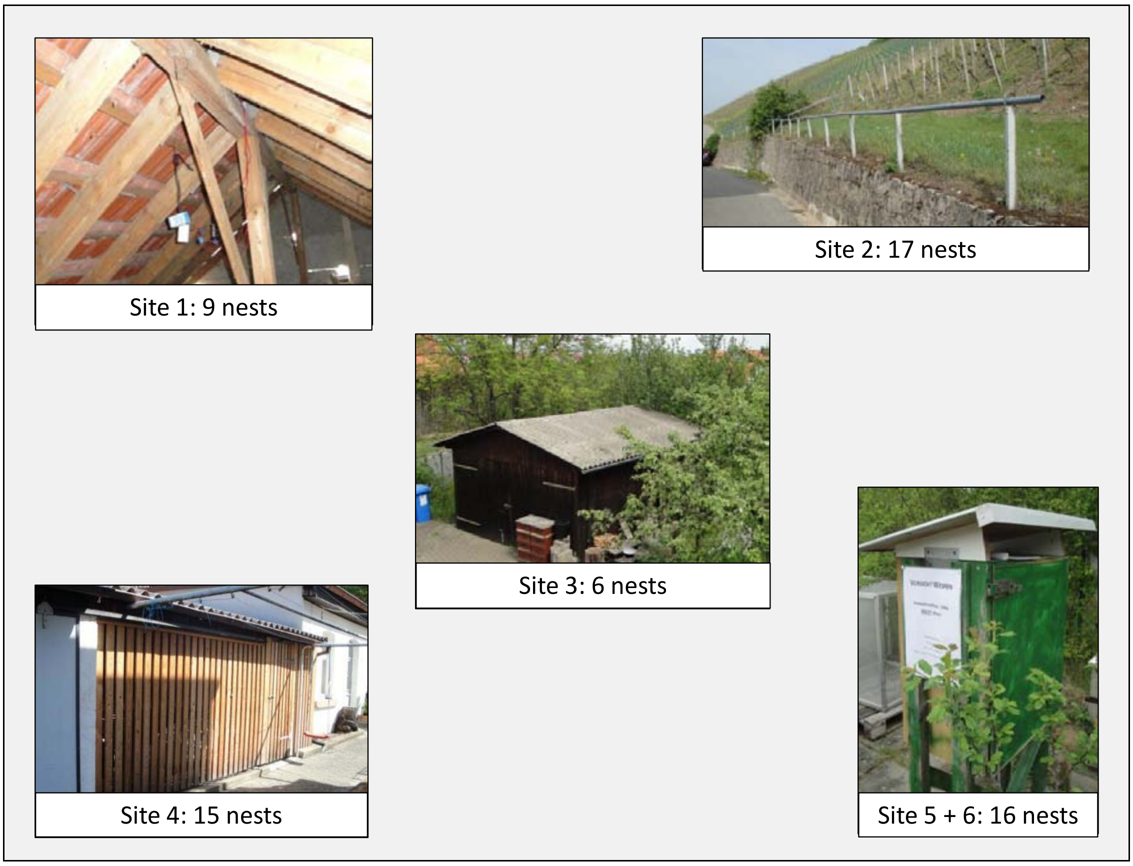


Fig. 12: Histogram of a total of 147 abandoned nests of *P. dominula,* detected upon four consecutive winter seasons (2011-2014) in the vicinity of Würzburg, Germany.
Nest sizes are classified according to their number of cells. Indicated are eight groups of increasing size each comprising of 30 cells.

4.1.2 Length of nesting season and nest sizes

The study on newly founded nests was conducted at six sites in and near Würzburg (within a 5.5 km radius) in spring and summer of 2012, 2013 and 2014 **(Fig. 11)**. In Germany, *P. dominula* colonies are be found almost exclusively in man-made structures (Blüthgen 1956, Bleidorn et al. 2000). Due to this fact, different nesting sites were selected. Within the three study seasons, nine nests were discovered under a rooftop with non-isolated tiles (site 1). Another location was a metal tube (5.5 cm diameter) at a vineyard (site 2). There, 17 nests were detected. Two other sites were under roofs built of corrugated eternit panels in barns (site 3 and 4). Inside those barns, a total of 21 nests were found. The last sites were two wooden boxes (58 x 52 x 31 cm), originally built for the breeding of wild bees (site 5 and 6). In the three consecutive seasons of 2012, 2013 and 2014 29, 16 and 18 nests, respectively, were observed **(Fig. 13)**.



Site 1: 9 nests

Site 2: 17 nests

Site 3: 6 nests

Site 4: 15 nests

Site 5 + 6: 16 nests

Fig. 13: Six nesting sites at which the nesting behaviour of *P. dominula* was investigated in more detail during the spring and summer seasons of three consecutive years.

At these six different locations, a total of 63 newly founded nests by *P. dominula* were monitored once a week during the whole summer seasons of 2012, 2013 and 2014 **(for details see Table S1)**. Noticeably, we observed a considerable difference in the total number and size of nests within the three years of our study **(Table 5)**. During the first year, we found 29 nests. In the following year, we could only detect 16 nests at the same sites. In the third year, we found 18 nests at our study sites.

Table 5: Basic principles of nesting of *Polistes dominula* wasps in the vicinity of Würzburg, Germany.

Year		**2012**	**2013**	**2014**	**2012- 2014** [a]
Length of nesting season [weeks]		18	20	21	19.7 (±1.5)
Number of nests	***Single***	12 (41%)	10 (62%)	12 (67%)	∑ 34 (54%)
	Multiple	17 (59%)	6 (38%)	6 (33%)	∑ 29 (46%)
Survival of colonies	***Single***	5 of 12 (42%)	4 of 10 (40%)	7 of 12 (58%)	16 of 34 (47%)
	Multiple	14 of 17 (82%)	5 of 6 (83%)	4 of 6 (67%)	23 of 29 (79%)
Number of cells at the end of founding	***Single***	56.5 (±24.1)	27.3 (±8.6)	48.3 (±6.9)	44.9 (±17.1)
	Multiple	61.4 (±17.3)	33.6 (±14.1)	67.3 (±19.2)	53.7 (±21.1)
Number of cells at the end of season	***Single***	81.5 (±19.6)	37.5 (±5.7)	115.1 (±20.8)	85.5 (±37.1)
	Multiple	82.1 (±22.06)	42.1 (±11.8)	132.3 (±17.0)	77.5 (±35.1)
Number of pupae at the end of founding	***Single***	10.8 (±8.4)	3.8 (±0.5)	6.3 (±4.5)	6.8 (±5.6)
	Multiple	10.9 (±5.5)	4.6 (±3.8)	8.3 (±4.8)	8.6 (±5.3)

The data are indicated separately for single and multiple founded colonies.

[a] This column displays mean values and standard deviations (in parentheses), except the number of nests which is represented by sums and survival rates which are shown by ratios and percentages (in parentheses)

The beginning of nesting of paper wasps strongly depends on the outside temperature. In 2012, the nest founding started in early April (calendar week = CW 14). During CW 23, the workers began emerging (= end of founding period). In CW 27, the last cells were built (= end of building phase) and the last reproductive individuals left the nest in CW 32 (= end of season). In 2013, the start of the nesting phase (CW 16 – 17) was delayed by a long cold period at the end of March. The end of the founding period was recorded in CW 28. The end of the building phase was detected in CW 31 and the season ended in September (CW 36). In 2014, nest founding started after an unusual warm winter, in CW 13, end of founding was reached in CW 22, end of building was recorded in CW 32 and the season ended in CW 34. During the three observation seasons, we observed an average length of the nesting season of 19.7 weeks **(Table 5). Figure 14** shows the progress of growth of nests per week during the three years. The end of founding was marked for each year by an arrow within the graph.

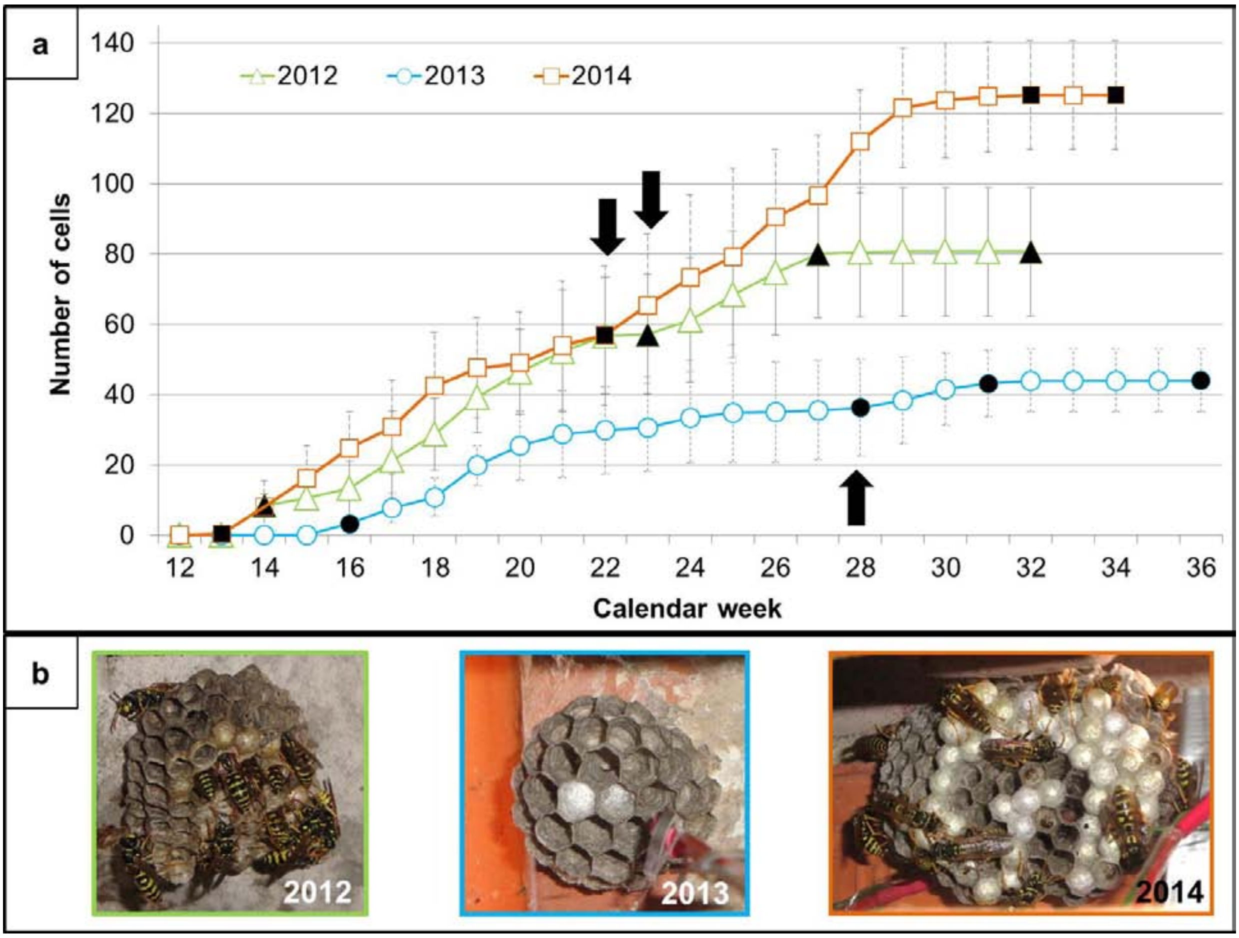


Fig. 14: Growth of newly founded *P. dominula* nests during 2012, 2013 and 2014 (CW 12 - CW 36).

a) The increase of cells per week of 29 nests (mean ± SD) is shown during the observation time from April 2 (CW 14) to August 12 (CW 32) of 2012 (△), of 16 nests (mean ± SD) between April 16 (CW 16) to September 8 (CW 36) of 2013 (○) and of 18 nests (mean ± SD) between March 24 (CW 13) to August 24 (CW 34) of 2014 (□). Closed triangles, circles and squares (▲ , ● , ■) mark: 1) the begin of the nest founding; 2) the end of founding; 3) the end of the building phase and 4) the end of the season. Black arrows (CW 23 in 2012; CW 28 in 2013; CW 22 in 2014) highlight the end of the founding period. b) Respective photographs of sample nests underline the different sizes of nests at the end of building.

The nests in 2013 were much smaller than in 2012, whereas the nests of 2014 were much bigger than in 2012. In 2012, they achieved a mean size of 80.7 cells (± 18.3); the nests in 2013 only attained sizes of approximately 50% of that (44.0 ± 9.0). In 2014, the nests achieved sizes of 125.2 cells (± 15.5), which means an enlargement of around 55% of the nests in comparison to 2012. During the three observation seasons, it became apparent that nests consisting of less than 31 cells were mostly given up during the founding period. Furthermore, I never recorded a newly founded nest bigger than 150 cells at the end of the season. The remarkable differences during the nesting seasons between the three years of observation can be explained by different temperatures **(Table 6)**. An extremely long and cold winter season was recorded in most parts of Germany in 2012/2013, whereas the winter 2013/2014 was unusual warm.

Table 6: Outside temperatures [°C] for Würzburg, Germany, per study year (modified from the Deutsche Wetterdienst = DWD).

Year	**2012**			**2013**			**2014**		
Months	**Max.**	**Average**	**Min.**	**Max.**	**Average**	**Min.**	**Max.**	**Average**	**Min.**
January	5.2	2.6	0.2	2.9	1.1	-1.0	6.1	3.4	1.0
February	1.8	-2.0	-5.5	2.2	-0.1	-2.3	8.7	4.8	1.4
March	13.3	8.2	3.1	6.1	1.9	-1.7	14.2	8.2	2.7
April	15.0	9.6	4.3	14.5	9.6	5.4	18.0	12.4	7.0
May	22.5	16.5	10.5	17.0	12.3	8.4	18.8	13.4	8.3
June	23.1	17.3	12.0	22.2	17.1	11.8	24.3	17.9	8.3
July	23.9	18.7	13.8	27.7	21.3	14.8	26.3	20.3	14.8
August	27.0	20.5	14.1	24.9	18.8	13.2	22.5	16.9	12.1
September	21.9	15.0	8.9	19.1	14.3	10.2	20.3	15.5	11.5
October	13.5	9.0	5.0	15.2	10.8	7.0	16.3	12.2	8.9
November	8.0	5.4	2.9	7.5	4.9	2.4	8.8	6.3	3.9
December	5.0	2.6	0.0	6.3	3.5	1.0	5.3	3.3	0.8

Late winter months (February and March), when the paper wasp queens slowly awake from hibernation and when they slowly start flying, strengthen themselves and start searching suitable nesting sites are as important as the months of nesting (April-September) itself. In Würzburg (Germany), February 2014 was much warmer than February 2012 (4.8°C vs. -2.0°C). The average temperature of March 2014 was comparable to March 2012. However, these temperatures were much higher (8.2°C) than in March 2013 (1.9°C). The temperatures of April 2012 and 2013 were similar (9.6°C), but April 2014 was warmer than in the other years (12.4°C). May 2013 and

2014 were 3-4°C cooler than May 2012, whereas the month of June was comparable in all three years. Again, in 2013 and 2014 July was warmer, August cooler and September comparable with the corresponding months in 2012 **(Table 6)**.

4.1.3 Foundress associations

At our six study sites, the number of foundresses per nest ranged between 1 and 4. The ratio between multiple- and single-foundress colonies was 29:34 over all three study years. This means that we were able to observe 46% multiple- and 54% single-foundress nests in our observation area. The difference of the ratio per year was also noteworthy. In 2012, we found more multiple- than single-founded colonies (17:12). In contrast, we were able to detect more single- than multiple-founded colonies in the following two years, i. e. 6:10 in 2013 and 6:12 in 2014 **(Table 5)**.

4.1.4 Survival rate of *Polistes dominula* nests

As described in the preceding chapter, the ratio of single- to multiple-foundress nests differed in consecutive years. Noteworthy, colony failure was much higher in single- than in multiple-foundress colonies **(Fig. 15)**. Only 16 of 34 (47%) single-foundress colonies survived, whereas 10 of 16 (63%) colonies founded by two queens survived. None of the observed nests, which were founded by more than two queens (n = 13) failed (df = 2; $X^2 = 11.2$; p = 0.004). Again, there are wide variations between the different observation seasons **(Table 5)**. During the first two years (2012 and 2013), survival rate of multiple-founded nests was about 80%, but in 2014, this rate was only 67%. For single-founded colonies, the opposite was the case. In the years 2012 and 2013, a survival rate of approximately 40% was documented, whereas the rate was elevated to 58% during the last year (2014).

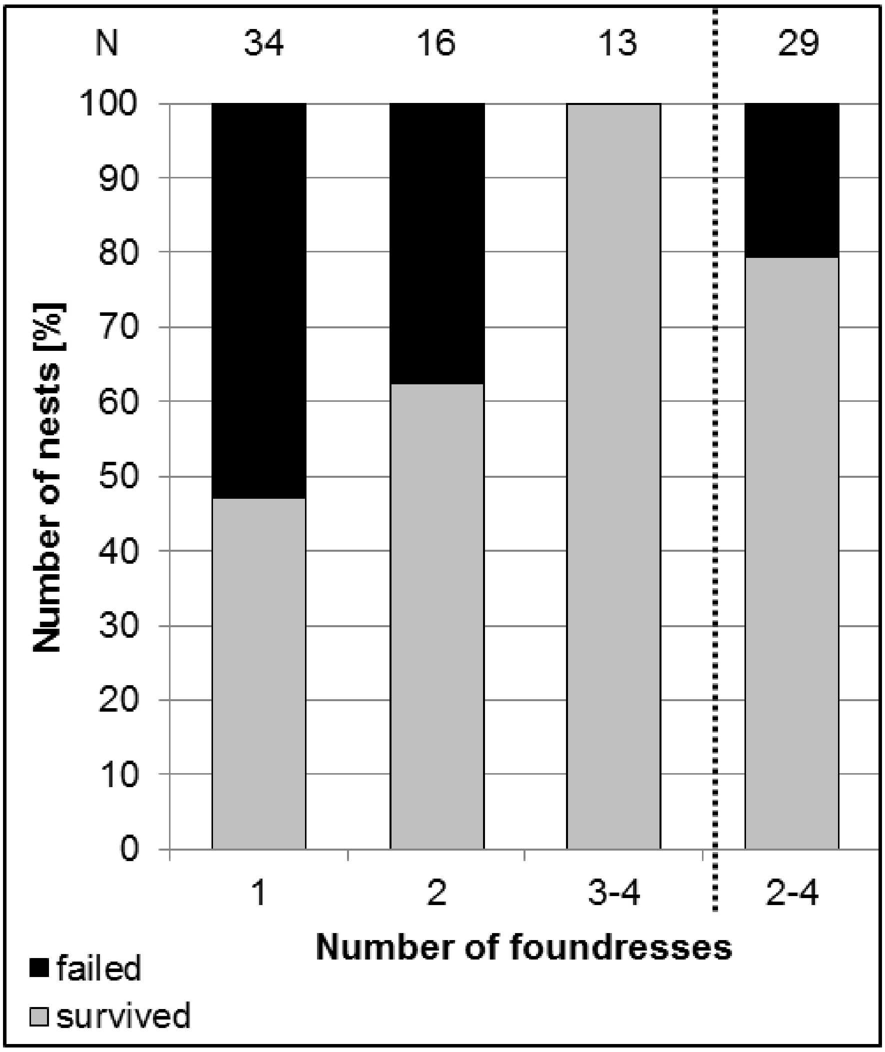


Fig. 15: Survival of *P. dominula* nests according to the group size of foundresses.
Light grey bars indicate the percentage of number of nests that survived and black bars indicate the number of nests that failed upon the observation period (summer seasons 2012, 2013 and 2014). The numbers above the bars show the distribution of group sizes (n = 63). The survival rate is shown for single (number of foundresses=1) and multiple founded (number of foundresses = 2-4) colonies, but also separately for nests that were founded by two queens and more than two queens (number of foundresses = 3-4).

4.1.5 Productivity of *Polistes dominula* nests

In order to test the productivity of colonies built by either a single or more than one queen, the number of cells and pupae were counted at the end of the founding period. In addition, the number of cells was counted again at the end of the season. Over all three years, no statistical difference was found between the numbers of cells at the end of the founding phase (Mann-Whitney test; $U = 135$; $p \geq 0.05$; $N_{(multiple)} = 26$; $N_{(single)} = 15$). Single founded colonies were composed of 44.9 cells (± 17.2). Nests founded by more than one queen had a mean number of 53.7 cells (± 21.1) at the same time.

At the end of the season, the nests founded by a single queen were composed of 85.5 cells (± 37.1) and the multiple-foundress nests of 77.6 cells (± 35.1). A Mann-Whitney test showed no statistical difference between single and multiple founded nests in number of cells at the end of the season ($U = 169.5$; $p \geq 0.05$; $N_{(multiple)} = 26$; $N_{(single)} = 15$). When we take a more detailed look at the values of the individual years, it is obvious that the mean size of the nests does not depend on the number of foundresses, but all the newly founded nests in 2013 reached only half the size of those nests in 2012. However, the nests in 2014 were on average three times larger than in 2013 **(Table 5; Fig. 16 A, C and E).**

The number of pupae at the end of the founding period in nests built by single or multiple foundresses was on the average nearly the same, too: Over all three years we found a mean number of pupae of 6.8 (±5.6) in single founded nests and 8.6 (±5.5) in multiple founded colonies. From the statistical point of view, no significance is detectable (Mann-Whitney test, $U = 147$; $p \geq 0.05$; $N_{(multiple)} = 26$; $N_{(single)} = 15$). A closer look at the data of the individual years showed nearly the same effect as described above. The number of pupae of the years 2012 and 2014 does not differ very much, but the number of pupae in 2013 is reduced by a factor of two in comparison to the other two years of study **(Table 5; Fig. 16 B, D and F)**.

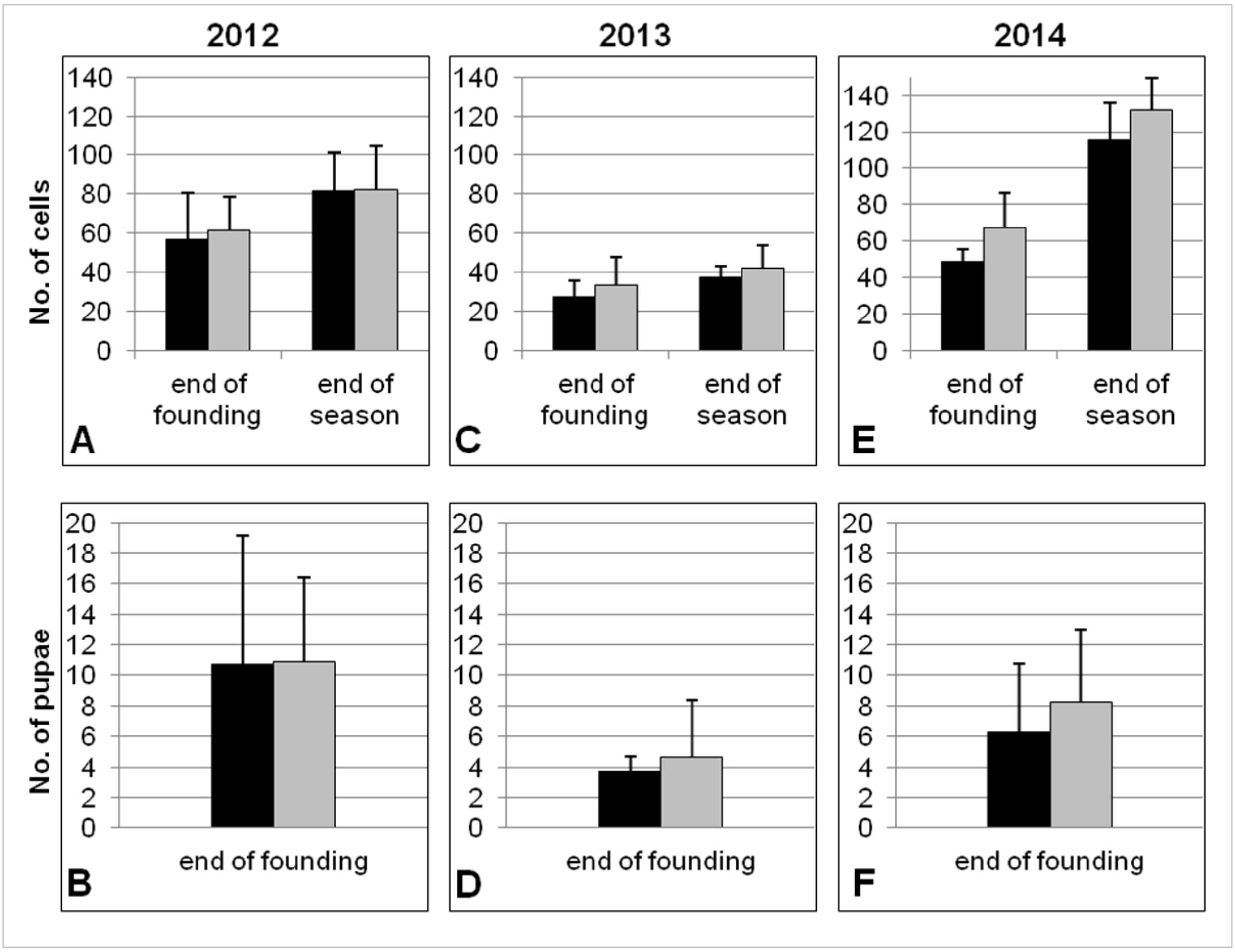


Fig. 16: Average sizes of nests and productivity of single (black bars) and multiple (light grey bars) founded *P. dominula* colonies at two different developmental times of the summer seasons 2012, 2013 and 2014.

(A) In 2012 ($n_{single} = 5$; $n_{multiple} = 14$), the number of cells is indicated for the end of the founding period (= CW 23) and for the end of the season (= CW32). (C) In 2013 ($n_{single} = 4$; $n_{multiple} = 5$), the number of cells is shown for CW 28 (= end of founding) and for CW36 (= end of season). (E) In 2014 ($n_{single} = 7$; $n_{multiple} = 4$), the number of cells is indicated for CW 22 (= end of founding) and for CW 34 (= end of season). (B, D, F) The number of pupae is specified for the end of the founding period of 2012 (CW 23), of 2013 (CW 28) and of 2014 (CW 22), respectively. Shown are the means ± SD; CW = Calendar week; consider the different y-axes.

4.2 THERMOREGULATORY MECHANISMS OF INDIVIDUAL ADULT PAPER WASPS

4.2.1 The examination of different artificial stressors for investigating the thermal behaviour of *P. dominula* wasps

Eleven colonies were selected for studying thermoregulation of individual *P. dominula* wasps. The thorax temperatures of adult wasps were measured with a thermographic camera before, during and after being exposed to two different stressors (smoke and CO_2) and to an air blast (negative control). It was decided to apply the stressors for three seconds only, because the wasps responded to a longer application of smoke with moving towards the exit of the sheltered nesting sites and fled. On the other hand, by the application of CO_2 for longer than three seconds or in a fitful way (simulating the natural breathing of a predator), the wasps hectically moved around, started flying and circled around the nest without trying to escape. The response of the investigated individuals is highlighted in **Figure 17**. The pictures of the thermographic shots clearly revealed a rise of the thorax temperatures after the wasps were stressed with smoke **(Fig. 17 b)** or CO_2 **(Fig. 17 d)** but not after the application of an air blast **(Fig. 17 f).**

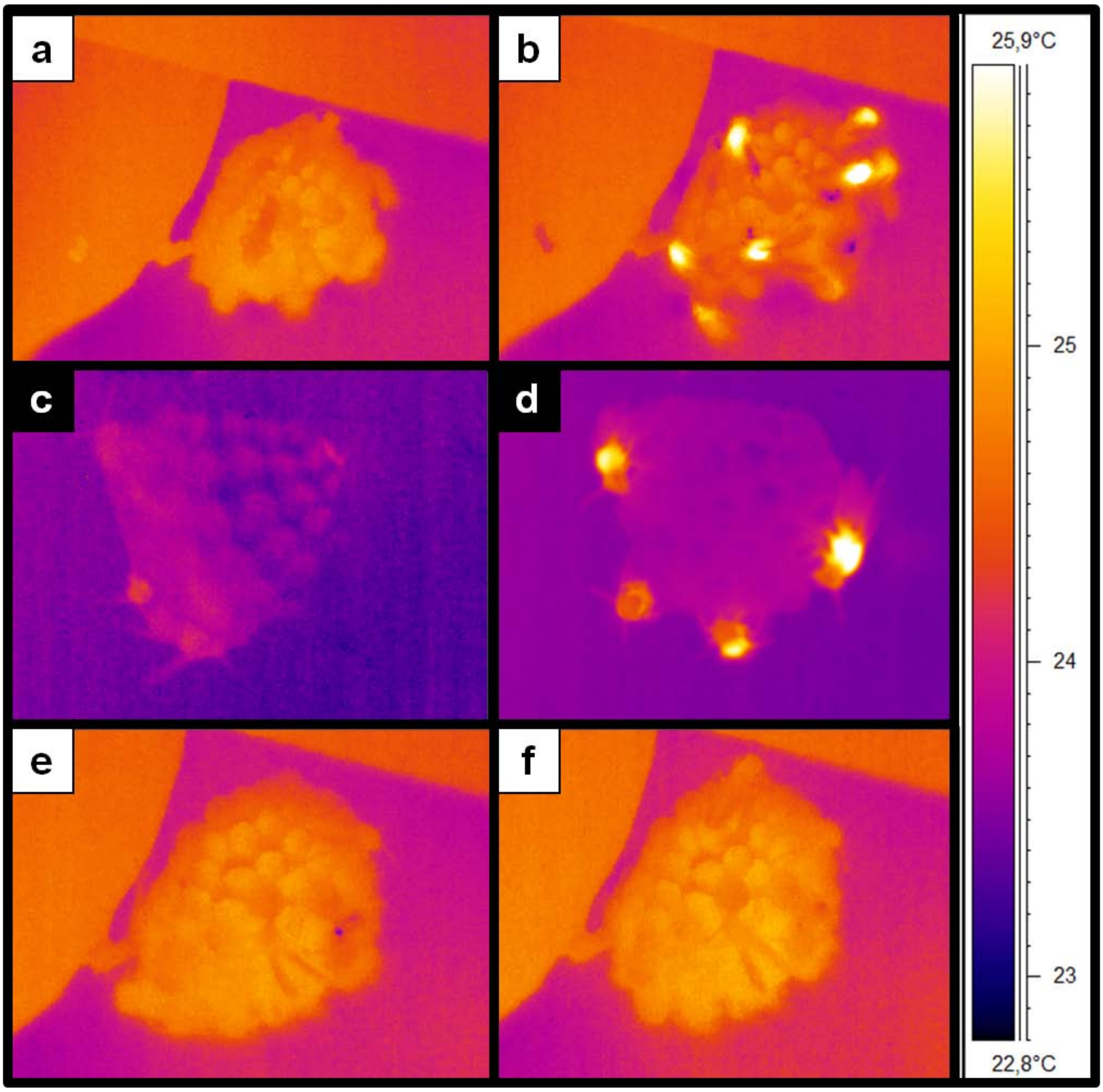


Fig. 17: Thermographic images of *P. dominula* nests before and after challenge with diverse stressors.

Pictures were taken with an infrared camera from different nests during the season when more than four workers had emerged. Images are presenting selected monitoring points: 10 s before (a) and 50 s after (b) the application of smoke (nest no. 13, site 3: July 15, 2012); 20 s before (c) and 60 s after (d) the application of CO_2 (nest no. 44, site 5: July 31, 2013); 10 s before (e) and 60 s after (f) the wasps were stressed with an air blast (nest no. 13, site 3: July 2, 2012). Temperature scales of the infrared images are represented at the right side.

4.2.2 Response of *Polistes dominula* to the different artificial stressors smoke and CO_2

The analysis of a single measurement of the wasps challenged with the stressor “smoke” is presented in **Fig. 18.** At monitoring point 10 s, the thorax temperature of the wasps was nearly the same as the nest temperature. The wasps started with an anxious behaviour at monitoring point 70 s, only 10 s after the stressor smoke was applied. The highest elevated thorax temperature were measured between monitoring points 120 s and 150 s with a mean increase of the thorax temperature of nearly 2°C **(Fig. 18 a)**. This is also illustrated by the respective thermographic image at monitoring point 140 s **(Fig. 18 b).** After this peak was reached, the temperature of the thoraces dropped.

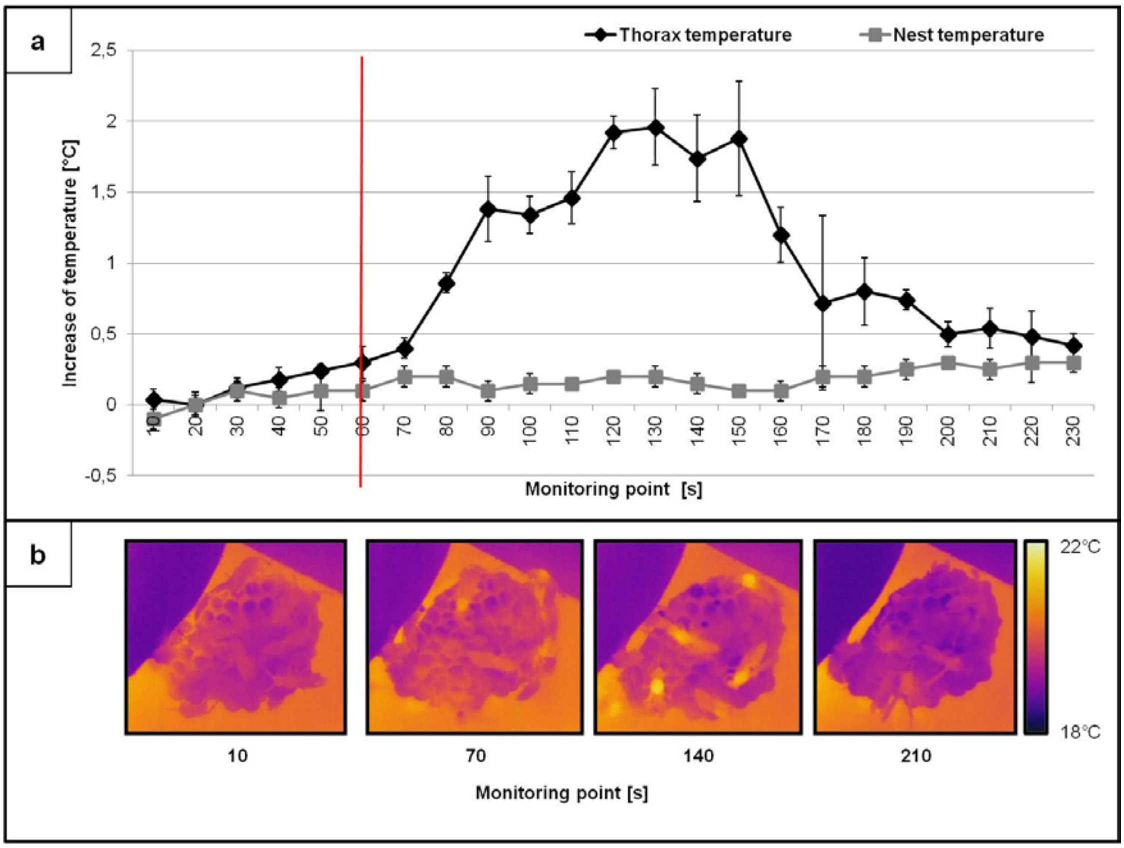


Fig. 18: Thorax temperatures of *P. dominula* wasps and the respective nest temperature during the challenge with smoke.
(a) A single stress situation with smoke is shown that was recorded at July 15, 2012 (nest no. 13, site 3). At that time, the nest consisted of 90 cells and 8 adult wasps. Pictures were taken every 10 s with an infrared camera for a total time interval of 230 s. (b) Thermographic images at selected monitoring points. Eight resting wasps are staying on the comb. Temperature scales of the infrared images are shown at the right side.

A further single measurement of the wasps stressed by CO_2 is shown in **Fig. 19.** The analysis of this case showed that the thorax temperature of the wasps was nearly the same as the nest temperature at monitoring point 10 s. The wasps started to heat up their thoraces at monitoring point 80 s, only 10 s after the stressor CO_2 was applied. The highest elevated thorax temperature was measured at monitoring point 130 s with a mean increase of the thorax temperature of over 2°C **(Fig. 19 a)**. The "hot" thoraces are also illustrated by the respective thermographic image at monitoring point 150 s **(Fig. 19 b).** After this peak was reached, the temperature of the thoraces dropped.

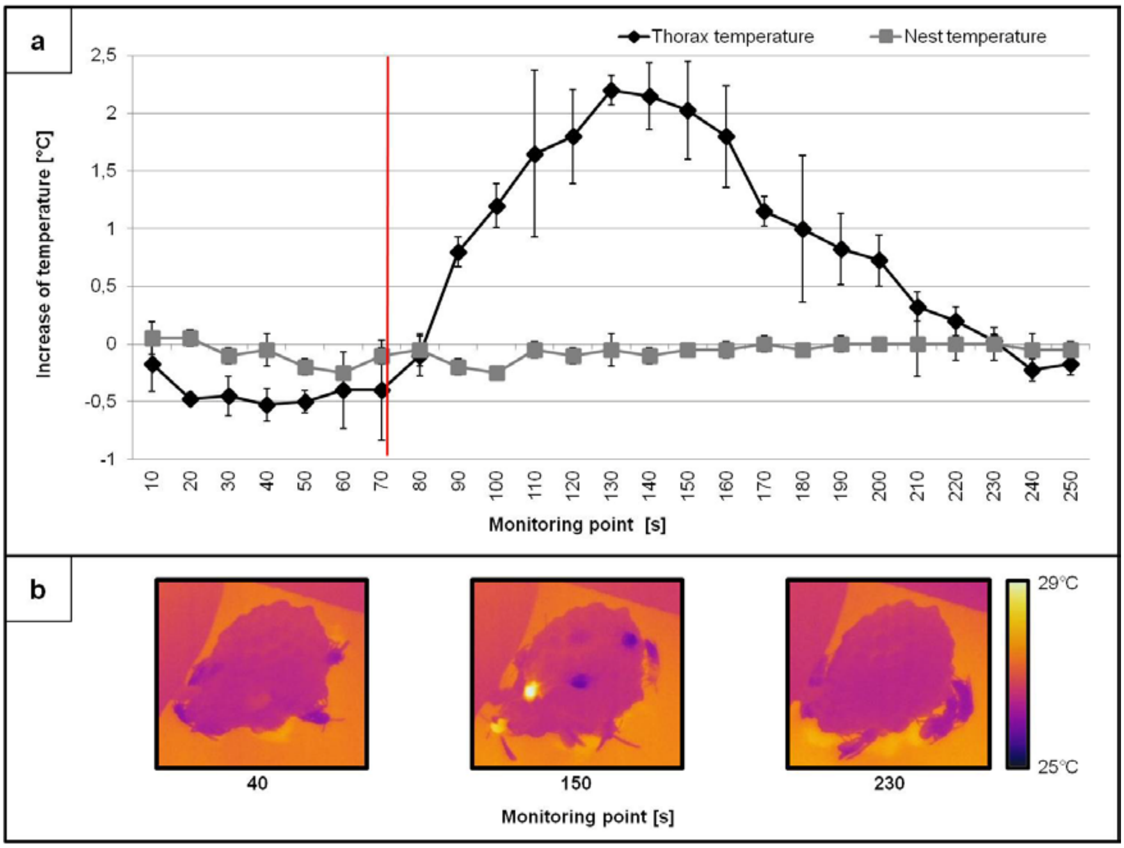


Fig. 19: Thorax temperatures of *P. dominula* wasps and the respective nest temperature during the challenge with CO_2.

(a) A single stress situation with smoke is shown that was recorded at August 7, 2012 (nest no. 13, site 3). At that time the nest consisted of 90 cells and 8 adult wasps. Pictures were taken every 10 s with an infrared camera for a total time interval of 250 s. (b) Thermographic images at selected monitoring points. Eight resting wasps are staying on the comb. Temperature scales of the infrared images are shown at the right side.

The comprehensive analysis of further single measurements revealed that the wasps responded to the stressor "smoke" by heating up their thoraces already 10 s after the smoke was applied and that after 50 s the highest thorax temperature was reached **(Fig. 20)**. The difference in thorax temperature to the state before the stressor was applied was 1.88°C (± 0.40). In case of CO_2, the wasps also showed a quick response to the stressor, 10 s after the application. However, it took 70 s until the maximum was reached (1.56°C ± 0.49). After that time, a gradual decline of the thorax temperatures was observed. The thoracic temperature of the air-treated wasps showed a slight increase directly after the air blast was applied to the animals. But with a mean increase of the thorax temperature of only 0.10°C (± 0.49), it seemed not to be a direct response to the air blast **(Fig. 20)**. This could be an effect of camera drift.

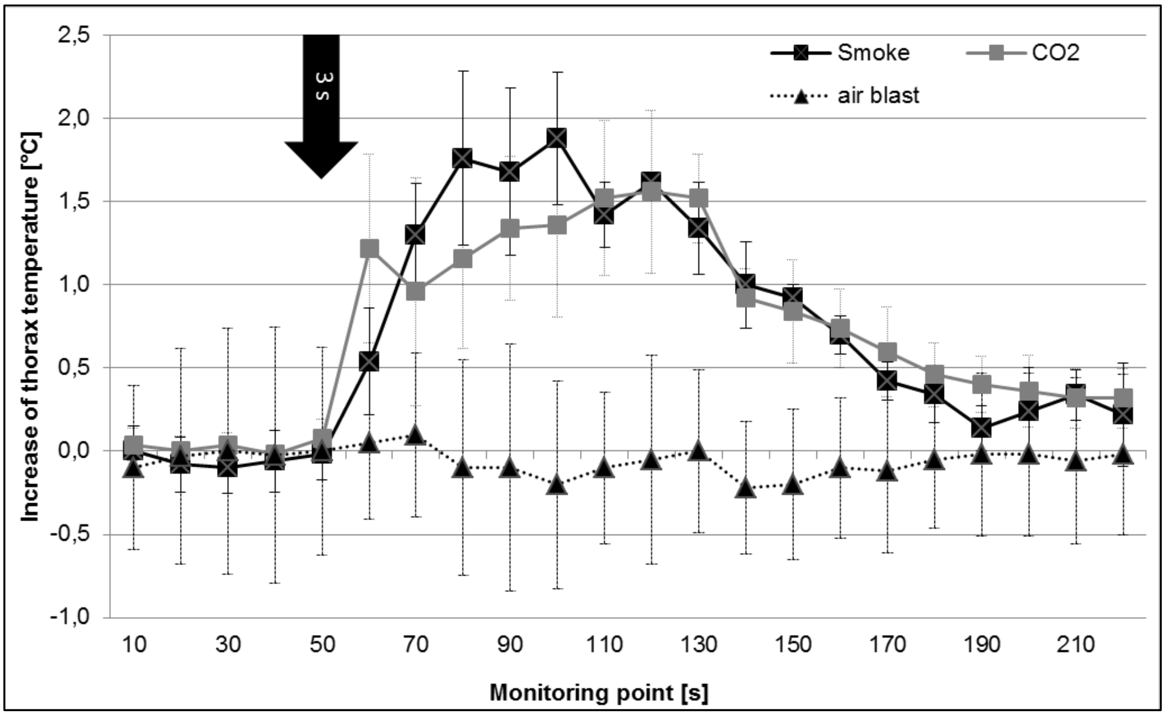


Fig. 20: Time-dependent recordings of thorax temperature of *P. dominula* wasps in response to the application of different stressors.
Single measurements are presented after challenge with smoke (nest no. 38, site 4: July 12, 2013) and with CO_2 (nest no. 13, site 3: August 6, 2012) or with air (nest no. 36, site 3: July 31, 2013). The black arrow indicates the point of time at which the corresponding stressor was applied (monitoring point 50 s). Infrared images were taken with a thermographic camera every 10 s. Bars represent the mean difference of thorax temperature (± SD) of four individual wasps.

Across all measurements, a similar picture emerged **(Table 7).** With an average time of 61.50 s (± 14.61), the adult wasps stressed with smoke attained the maximal thorax temperature slightly faster than the wasps stressed with CO_2 (65.00 s ± 6.88). Similarly, the mean increase of the thorax temperature itself was slightly higher in smoke-stressed wasps (1.90°C) as in CO_2-stressed wasps (1.60°C). The negative control, analysed 12 times, clearly revealed that the wasps did not react to the air blast. Furthermore, resting wasps were monitored, to ensure that no increase of thorax temperatures takes place under natural – untreated – conditions. The resting wasps showed no major changes in thorax temperatures during three minutes of analysing, comparable to the air blast-treated wasps **(Table 7).** In twelve measurements we could only detect a variation of the thorax temperature of 0.1°C (± 0.21).

Table 7: Summary of the response of *P. dominula* wasps to different stressors.

	N	**Increase of thorax temperature [°C] after applying the stressor**			**Time interval [s] to reach maximal thorax temperatures**		
		Maximum	**Minimum**	**Mean ± sd**	**Maximum**	**Minimum**	**Mean ± sd**
Smoke	20	2.66	1.26	1.91 ± 0.34	80	30	61.50 ± 14.61
CO_2	20	2.28	0.82	1.56 ± 0.49	80	50	65.00 ± 6.88
Air Blast	12	0.18	0.03	0.09 ± 0.05	-	-	-
Resting on the nest	12	0.22	0	0.10 ± 0.21	-	-	-

Before supplying the stressors, the three groups of wasps showed approximately similar basic thorax temperatures **(Fig. 21;** monitoring point 10 s). There was no statistical difference neither between smoke-stressed wasps and the negative control (air) (Mann-Whitney test; U = 116; $p > 0.05$; $N_{(smoke)} = 20$; $N_{(air)} = 12$), nor between CO_2-stressed wasps and the negative control (Mann-Whitney test; U = 77.5; $p > 0.05$; $N_{(CO2)} = 20$; $N_{(air)} = 12$), nor between smoke-stressed and CO_2-stressed wasps (Mann-Whitney test; U = 126; $p \geq 0.05$; $N_{(smoke)} = 20$; $N_{(CO2)} = 20$).

Ten seconds after the application of the stressors (monitoring point 60 s), the differences in thorax temperature were visible and statistically evident **(Fig. 21)**. The smoke- stressed wasps showed a rise of thorax temperature of about 0.48°C (± 0.36) compared to their basic thorax temperature. The reaction of the "CO_2-group" revealed an increase of thorax temperature of about 0.86°C (± 0.76). However, the wasps treated with air exhibited stable thorax temperatures (0.05°C ± 0.36). The statistical difference between smoke-stressed wasps and the negative control disclosed a p-value of 0.003 (Mann-Whitney test; U = 42; $N_{(smoke)} = 20$; $N_{(air)} = 12$). A further Mann-Whitney test also showed a significant difference between CO_2-stressed wasps and the negative control (U = 38.5; p = 0.002; $N_{(CO2)} = 20$; $N_{(air)} = 12$). No statistical difference was detectable, between smoke-stressed and CO_2-stressed wasps (Mann-Whitney test; U = 142; p = 0.119; $N_{(smoke)} = 20$; $N_{(CO2)} = 20$).

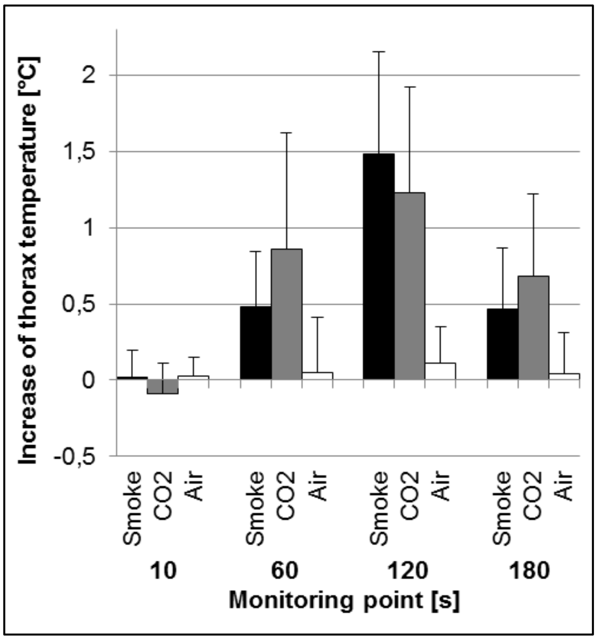


Fig. 21: Mean differences in thorax temperatures of *P. dominula* wasps after the application of different stressors.

Bars represent the mean difference in thorax temperature (± SD) across all measurements ($N_{(smoke)} = 20$; $N_{(CO2)} = 20$; $N_{(air)} = 12$). Before applying the stressors, all wasps exhibited a comparable thorax temperature (monitoring point 10 s). After the application, the thorax temperatures of smoke- and CO_2-treated wasps rose continuously within the next two minutes (monitoring points 60 and 120 s) and then gradually decreased (monitoring point 180 s). Application of air blasts had no effect on thorax temperatures of the treated wasps.

One minute later (monitoring point 120 s), the differences of the reactions to the stressors smoke and CO_2 with the negative control had increased **(Fig. 21).** At this point, the group of the smoke-stressed wasps had the highest rise in mean thorax temperature (1.48°C ± 0.67). Similarly, the CO_2-stressed wasps showed an average increase of thorax temperature of 1.23°C (± 0.69) at the monitoring point. The air-treated wasps revealed a consistent average difference in mean thorax temperature (0.11°C ± 0.24). The values of the Mann-Whitney test for smoke-stressed wasps compared to the negative control were $U = 14$; $p = 0.00$; $N_{(smoke)} = 20$; $N_{(air)} = 12$. CO_2-stressed wasps compared to the air blast-stressed wasps showed the following values: Mann-Whitney test; $U = 8$; $p = 0.00$; $N_{(CO2)} = 20$; $N_{(air)} = 12$. There was no statistical difference between the response of smoke- and CO_2-stressed wasps (Mann-Whitney test; $U = 148$; $p = 0.165$; $N_{(smoke)} = 20$; $N_{(CO2)} = 20$).

About two minutes after applying the stressors, the elevated thorax temperatures had dropped (monitoring point 180 s). The smoke- and CO_2-stressed paper wasps showed an elevation in mean thorax temperature of 0.47°C (± 0.40) and 0.68°C (± 0.54), respectively. The negative control group again showed no reaction (0.04 ± 0.27). Using the Mann-Whitney test, a statistical difference was still detectable between the smoke- and CO_2-stressed wasps and the negative control ($U = 59$; $p = 0.009$; $N_{(smoke)} = 20$; $N_{(air)} = 12$; $U = 37$; $p = 0.0013$; $N_{(CO2)} = 20$; $N_{(air)} = 12$, respectively). Even at this point, no statistical difference between smoke- and CO_2-stressed wasps was detectable (Mann-Whitney test; $U = 153.5$; $p = 0.215$; $N_{(smoke)} = 20$; $N_{(CO2)} = 20$).

4.2.3 The thermal behaviour of dominant and subordinate *Polistes dominula* queens during dominance battles

The thorax temperatures of adult *P. dominula* queens were measured with an infrared camera during dominance battles within the founding phase of the season, i. e., before the emergence of the first workers. In all observed battles, the dominant queen did not initiate but responded to the attack of the subordinate queen. The analysis of a single dominance battle showed very impressively the different reactions with respect to thorax temperatures of the dominant and the subordinate queen **(Fig. 22 a).** Both involved queens showed nearly the same thorax temperatures at the beginning of the fight, with no sign of endothermy. After 40 s, while the battle had become more violent, the thorax temperature of the dominant queen rose very quickly. Within another 40 s, the highest thorax temperature was reached. In this specific case of a recorded battle, the dominant queen displayed an increase of thorax temperature of 1.60°C as compared to the basic temperature before the onset of the battle. On the other hand, the subordinate queen showed only minor changes of the thorax temperature during the whole fight **(Fig. 22 a).**

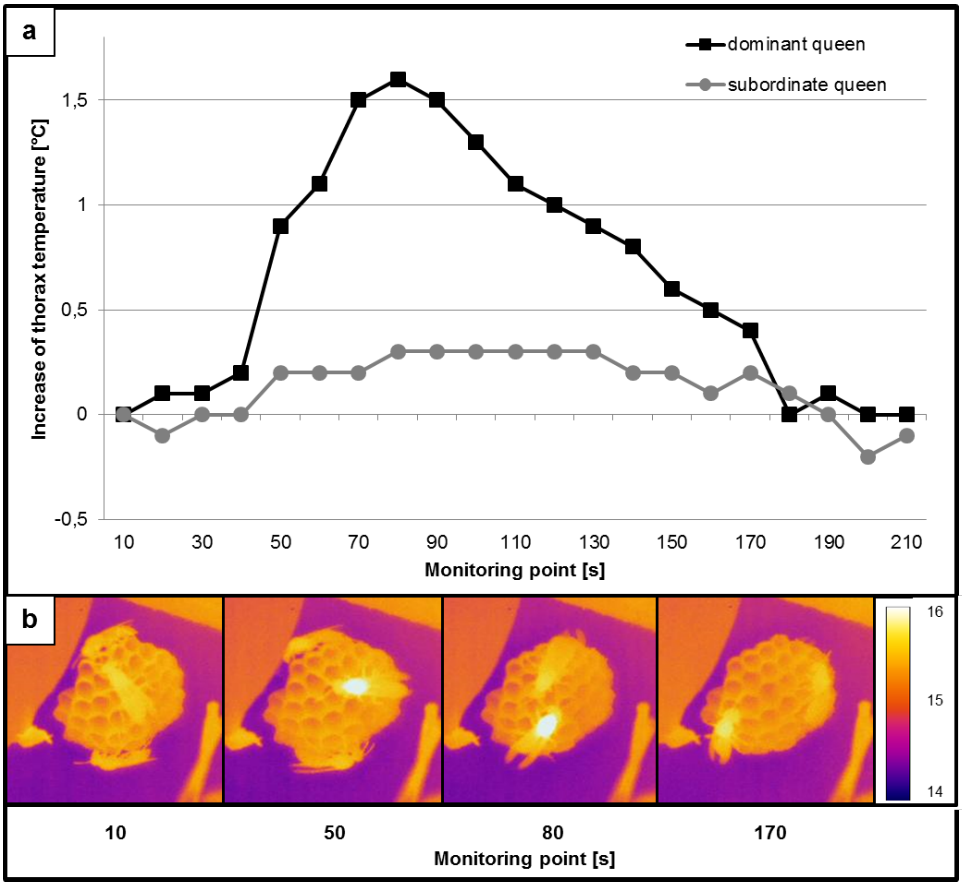


Fig. 22: Thorax temperatures of individual queens of *P. dominula* during the founding period.
(a) A single dominance battle is shown that was recorded at May 29, 2013 (nest no. 36, site 3). At that time the nest consisted of 48 cells. Pictures were taken every 10 s with an infrared camera for a total time interval of 210 s. (b) Thermographic images at selected monitoring points. Two subordinate queens staying on the edge of the comb and a dominant queen resting in the centre of the nest are visible. Temperature scales of the infrared images are shown at the right side.

The respective pictures of the thermographic recording of this dominance battle clearly illustrate a rise of the thorax temperature after the beginning of the dominance battle **(Fig. 22 b).** Ten seconds after the measurement started, the thorax temperatures of all three visible queens appeared approximately equal. After 50 s, the dominant queen, sitting directly on the comb, showed an enhanced thorax temperature. Another 40 s later, the difference is most obvious, whereas at the end of the fight both visible queens exerted nearly the same thorax temperatures again.

Across all 12 measurements of dominance battles, a heating up of the thorax of the dominant queens between 1.10°C and 5.20°C was observed. With a mean rise of thorax temperature of 2.23°C (± 1.35), the dominant queens displayed the highest reaction in this study during the fights. The subordinate queens displayed a mean rise of thorax temperature of only 0.46°C (± 0.32; max. 1.10°C; min. 0.1°C) during the twelve investigated battles. This difference between dominant and subordinate queen is also statistically significant (Mann-Whitney U-test; $U = 161$; $p = 0.015$; $N_{(battles)} = 12$).

4.3 THERMOREGULATORY MECHANISMS IN *POLISTES DOMINULA* NESTS

4.3.1 The comparison of nest temperature between active and non-active nests

4.3.1.1. Single thermographic shots of an active nest revealed first insights of nest temperatures

In order to get a first impression of nest temperatures during the day, a single nest (nest no. 1, site 1; see **Table S1**) was selected and filmed with an infrared camera at different times. **Figure 23** shows that the nest was up to 5.5°C colder than the bricks of the roof during the morning **(Fig. 23 a),** whereas the roof was about 2°C cooler at the evening **(Fig. 23 c)**. The respective pictures of the thermographic recordings clearly illustrate the differences in temperature between nest and roof **(Fig. 23 b and d)**. Furthermore, it became apparent that there is a temperature gradient within the nest. The edges of the nest are about 1°C warmer as the brood cells during the morning. In the evening, the edges of the nest are about 1°C warmer than the brood cells.

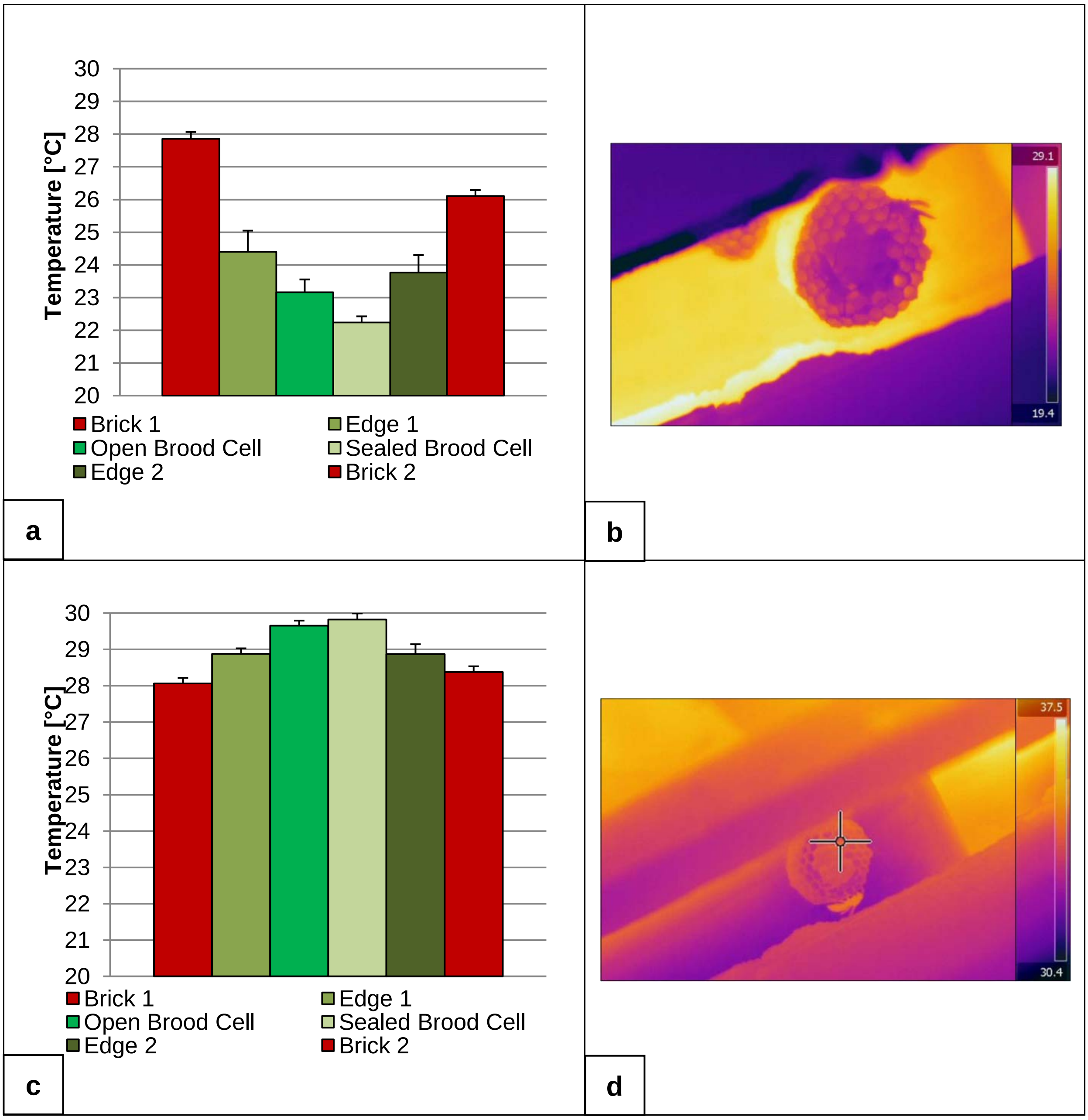


Fig. 23: Temperatures of a *P. dominula* nest at 10.00 am and 6.00 pm.
Single thermographic shots are shown that were recorded at May 28, 2012 (nest no. 1, site 1). At that time the nest consisted of 87 cells. Pictures were taken every 10 s with an infrared camera for 310 s at 10.00 am (a) and 6.00 pm (c). The respective thermographic images of the shot are shown at 10.00 am (b) and 6.00 pm (d). Temperature scales of the infrared images are shown at the right side of the respective picture.

4.3.1.2 Thermographic shots over 24h reveal differences in nest temperature between active and non-active nests

Because there were obvious differences in nest temperature, an experimental setup was established to investigate the nest temperature throughout the course of the day. In total, 29 thermographic shots were taken, 16 with (active nests) and 13 without (non-active nests) adult wasps on the nest. By recording active and non-active nests during similar outside temperatures, an indirect comparison was possible and gave a first indication of the influence of adult wasps on nest temperature. All 24 h series revealed the interesting observation that the nest and roof temperature was nearly the same at 8.00 am and 8.00 pm, regardless of the presence or absence of adult wasps on the nest and of outside temperatures.

The analysis of single monitoring days at low outside temperatures (15°C) showed that the active nest followed nearly the roof temperature even if it was on average 1.04°C (±0.29) colder than the roof during daytime (10.00 am – 6.00 pm) and 0.67°C (± 0.31) warmer at night (10.00 pm – 6.00 am; **Fig. 24 a)**. At 10 am and 4 pm the highest differences in temperature, 1.4°C and 1.3°C, respectively, were recorded.

One and a half month later, the same non-active nest showed differences at comparable outside temperatures **(Fig. 24 b)**. The nest temperature followed the outside temperature, but the nest was much warmer without wasps sitting on the nest. Between 10.00 am and 6.00 pm the nest was on average 1.31°C colder and between 10.00 pm and 6.00 am 1.53°C warmer than the roof. This result indicates that no active but a weak passive cooling takes place even on days of lower outside temperature.

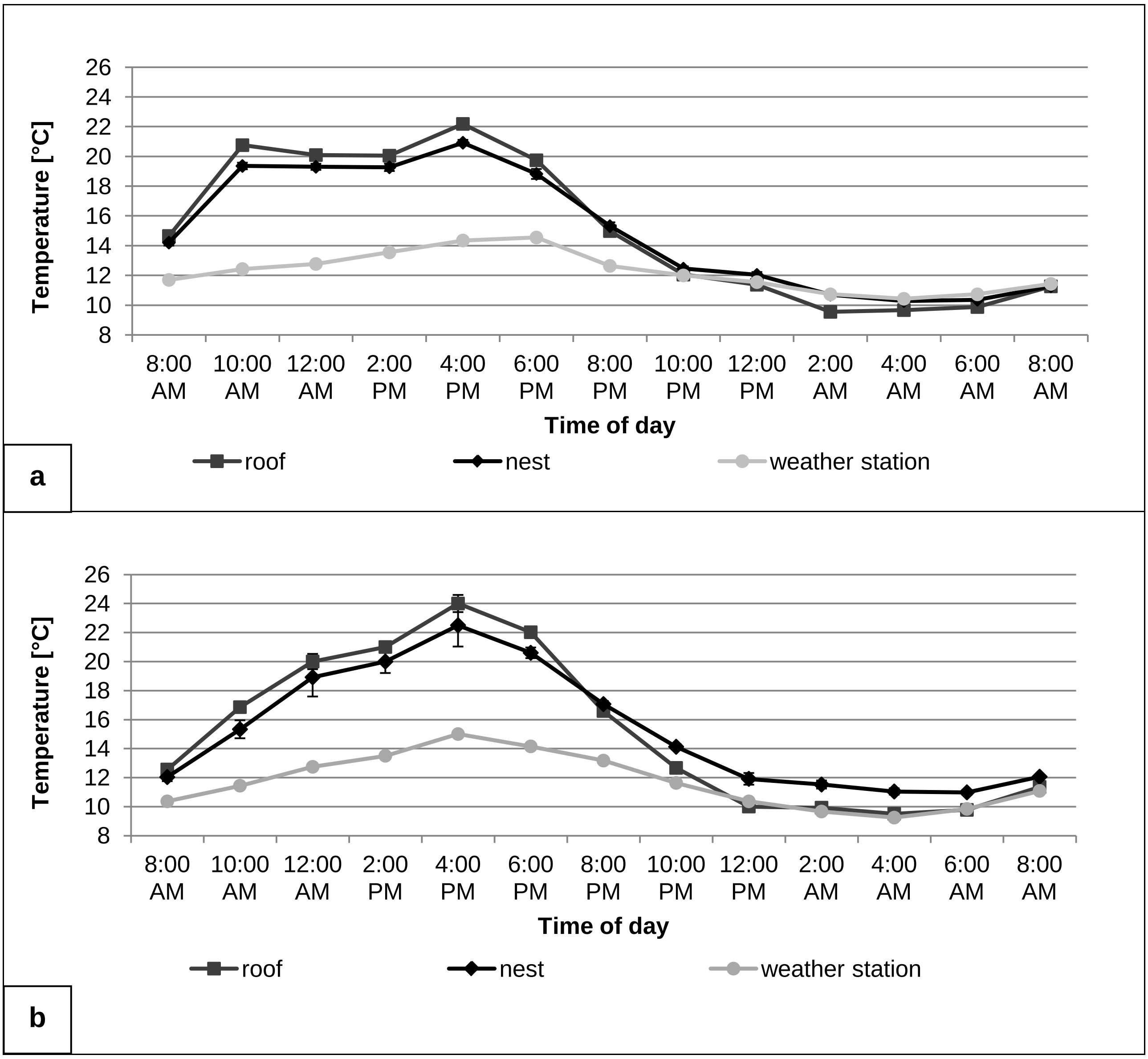


Fig. 24: Temperatures of *P. dominula* nests with (a) and without (b) adult animals, during 24 h of observation at a maximal outside temperature of 15°C.

Thermographic shots are shown that were recorded at (a) May 2, 2013 for the observation of nest temperature with adult wasps sitting on the nest and at (b) June 27, 2013 for a nest without wasps. Both shots were taken from nest no. 36 at site 3 **(see Table S1)**. Pictures were taken every two hours with an infrared camera for 310 s from 8.00 am to the next day 8.00 am. The weather station data represent the outside temperature. Means ± SD are represented for nest and roof temperatures.

The analysis of single monitoring days at high outside temperatures (35°C) showed that the active nest not longer followed the roof temperature during the day. It is on average 13.63°C (± 7.33) cooler than the roof during the day (10.00 am – 6.00 pm) and 0.67°C (± 0.35) warmer at night (10.00 pm – 6.00 am; **Fig. 25 a)**. At 10.00 am the difference in temperature was highest with 22.85°C. This result indicates that adult wasps exhibit a very effective cooling of the nest and that they are able to stabilize the nest temperature at 35°C which could be the optimal temperature for brood development.

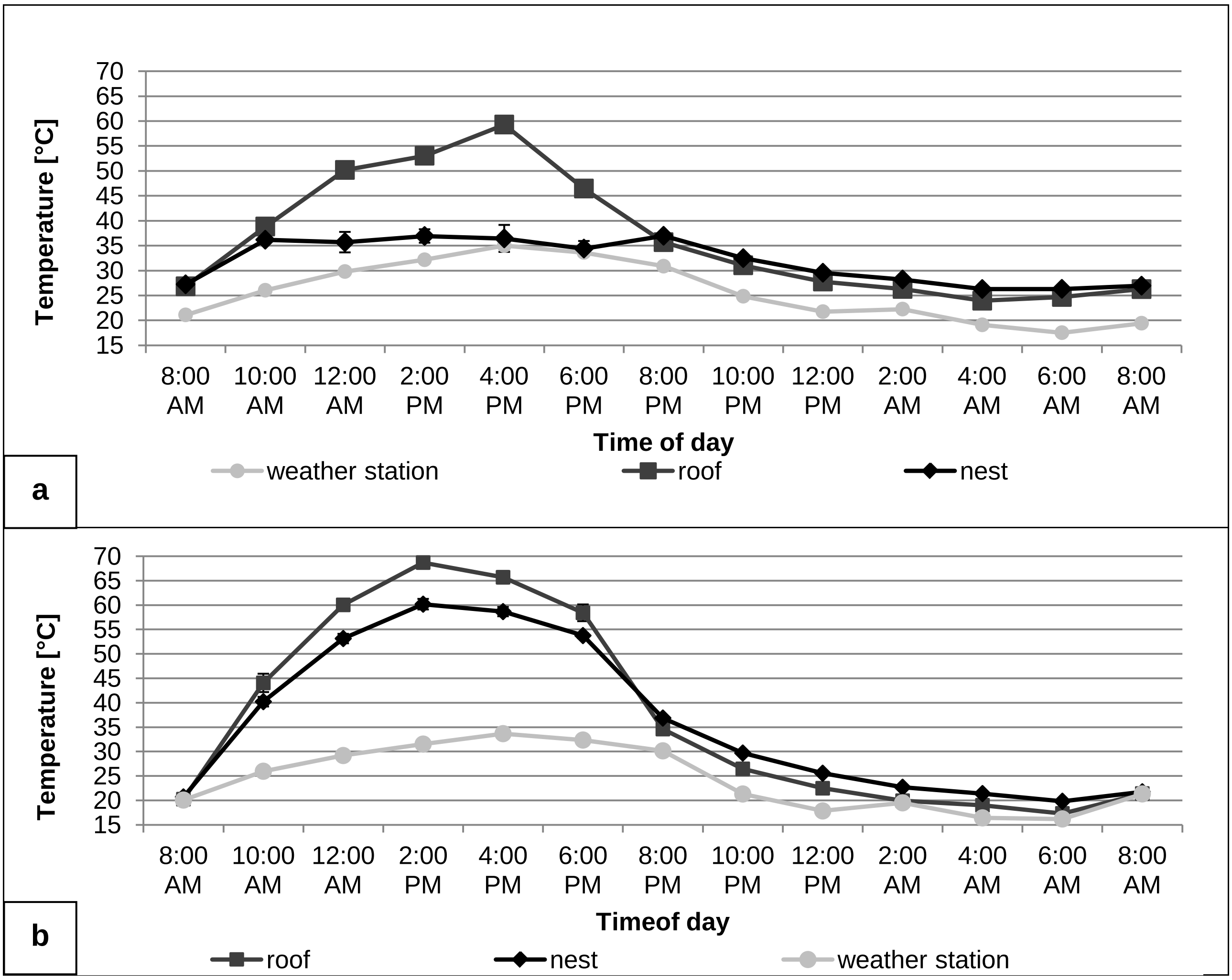


Fig. 25: Temperatures of *P. dominula* nests with (a) and without (b) adult animals, during 24 h of observation at a maximal outside temperature of 35°C.
Thermographic shots are shown that were recorded at (a) July 27, 2012 (nest no. 13, site 3) for the observation of nest temperature with adult wasps sitting on the nest and at (b) July 22, 2013 (nest no. 36, site 3) for data without wasps. Pictures were taken every two hours with an infrared camera for 310 s from 8.00 am to the next day 8.00 am. The weather station data represent the outside temperature. Means ± SD are represented for nest and roof temperatures.

The measurement of a non-active nest showed differences at comparable outside temperatures **(Fig. 25 b)**. The nest temperature followed the outside temperature, and the nest was much warmer without wasps sitting on the nest. Between 10.00 am and 6.00 pm the nest was on average 6.24°C (± 1.93) colder and between 10.00 pm and 6.00 am 2.87°C (± 0.37) warmer than the roof. This monitoring day clearly revealed that a kind of thermoregulation takes place even in non-active nests. At 4.00 pm, the difference between nest and roof temperature is 8.5°C. Since there is no active thermoregulation possible, this cooling effect can only be explained by a kind of passive thermoregulation.

4.3.1.3 Simultaneous monitoring of active and non-active nests at outside temperatures varying between 15°C and 35°C

As was demonstrated in the former chapter, no considerable thermoregulation of *P. dominula* nests took place during the night. Due to this fact, it was decided to revise the experimental setup. The differences to the earlier mentioned experiment **(see Figs. 24 and 25)** were a) that the data recording took place only during the day (between 8.00 am and 8.00 pm) and b) that the active and non-active nests were monitored simultaneously at the same nesting site to get convincing data sets of the influence of adult *P. dominula* wasps on the nest temperature.

a) Thermographic shots over 12h at a maximal outside temperature of 15°C

The analysis of one representative data set (out of n = 5) showed that the roof can reach a temperature of about 30°C even on a cool monitoring day with a maximal outside temperature of 15°C **(Fig. 26 a)**. Again, the nest temperatures of the active and non-active nest and the roof temperature are nearly the same at 8.00 am and 8.00 pm. Furthermore, the active and the non-active nest displayed almost identical temperatures throughout the recording. This indicates that no active thermoregulation was initiated by the adult wasps at a maximal outside temperature of 15°C. However, a certain passive thermoregulation was detectable. The active nest is on average 1.32°C (± 0.46) and the non-active nest 1.37°C (± 0.59) cooler than the roof between 10.00 am and 6 pm. The respective images of the thermographic recordings clearly illustrate the differences in temperature between the nests and the roof during the course of the day **(Fig. 26 b)**. The photo at 8am shows that the nests are slightly warmer than the roof, while at noon they are already visible cooler. At 4.00 pm this impression is reinforced, but at 8.00 pm, the nests reached the roof temperature again.

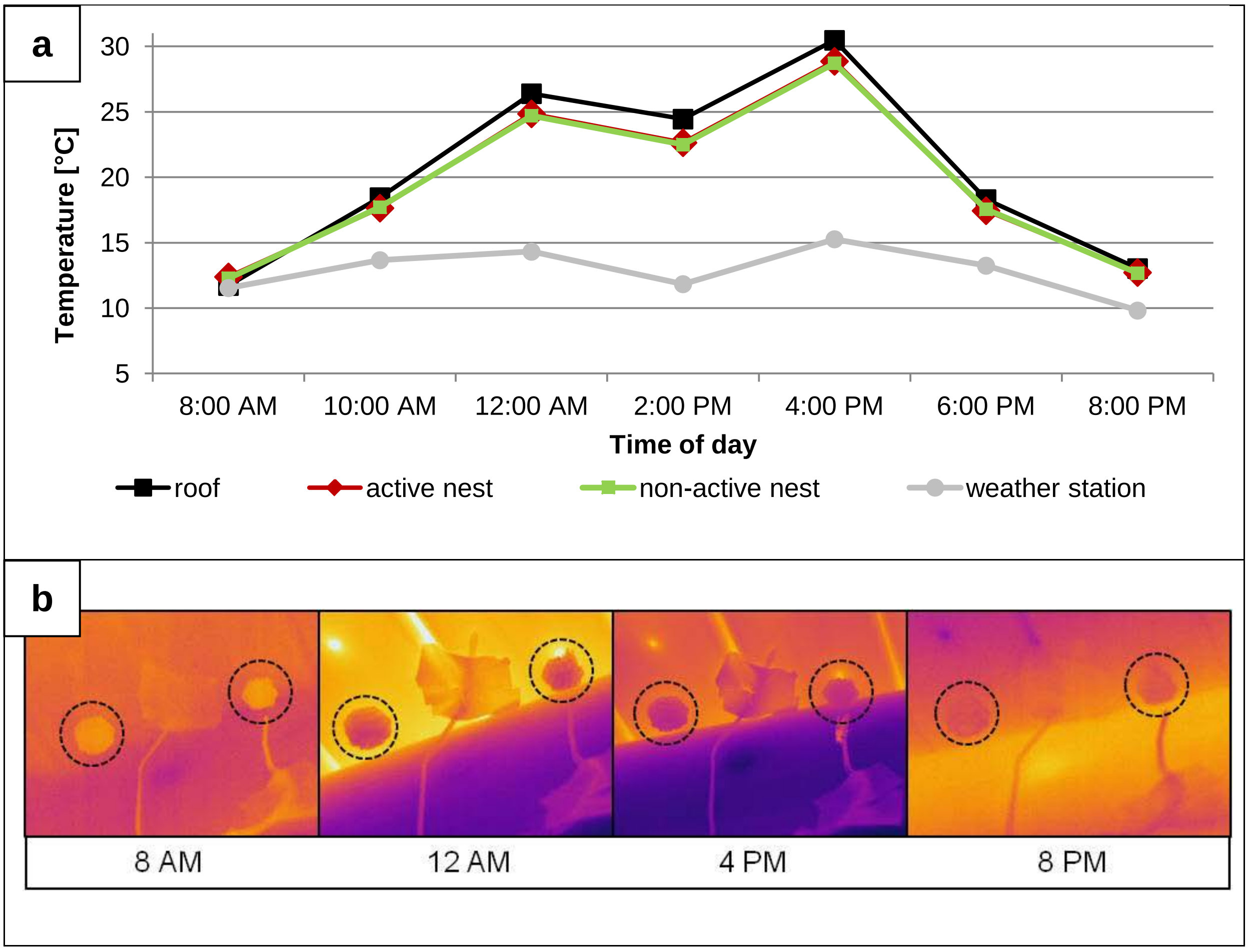


Fig. 26: Comparison of Temperatures of newly founded and old *P. dominula* nests at a maximal outside temperature of 15°C.
(a) Thermographic shots are shown that were recorded at May 2, 2014 (active nest no. 63; site 3; 30 cells; right side; **Table S1**/ non-active nest no. 13; 38 cells; left side; **Table S2**) for the observation of nest temperature. Means ± SD are represented for nest temperatures. Pictures were taken every two hours with an infrared camera for 310 s from 8.00 am to 8.00 am. (b) Thermographic images at selected monitoring points. Yellow/orange = higher temperature, purple/blue = lower temperature. The dotted black circles mark the nests.

b) Thermographic shots over 12h at a maximal outside temperature of 20°C

At a maximal outside temperature of 20°C, the analysis of one representative data set (out of n = 5) showed a similar picture as compared to an outside temperature of 15°C. The active and the non-active nest expressed equal temperatures throughout the recording over 12 h **(Fig. 27 a)**. This means that no active thermoregulation took place by the adult *P. dominula* wasps at outside temperatures of 20°C. However, a passive thermoregulation was again detectable. The active nest is on average 1.53°C (± 0.73) and the non-active nest 1.52°C (± 0.79) cooler than the roof between 10.00 am and 6.00 pm. Again, the nest temperatures of both nests and the roof temperature was nearly the same at 8.00 am and 8.00 pm.

The respective pictures of the thermographic recordings clearly illustrate the differences in temperature between the nests and the roof during the course of the day **(Fig. 27 b)**. The photo at 8.00 am shows that the nests are slightly warmer than the roof, while at noon they are already visible cooler. At 4.00 pm the nest temperatures approached the roof temperature, but at 8.00 pm the nests reached the roof temperature again. This single case implies that an increase of the outside temperature by 5°C must not necessarily lead to a warmer roof and nest temperature.

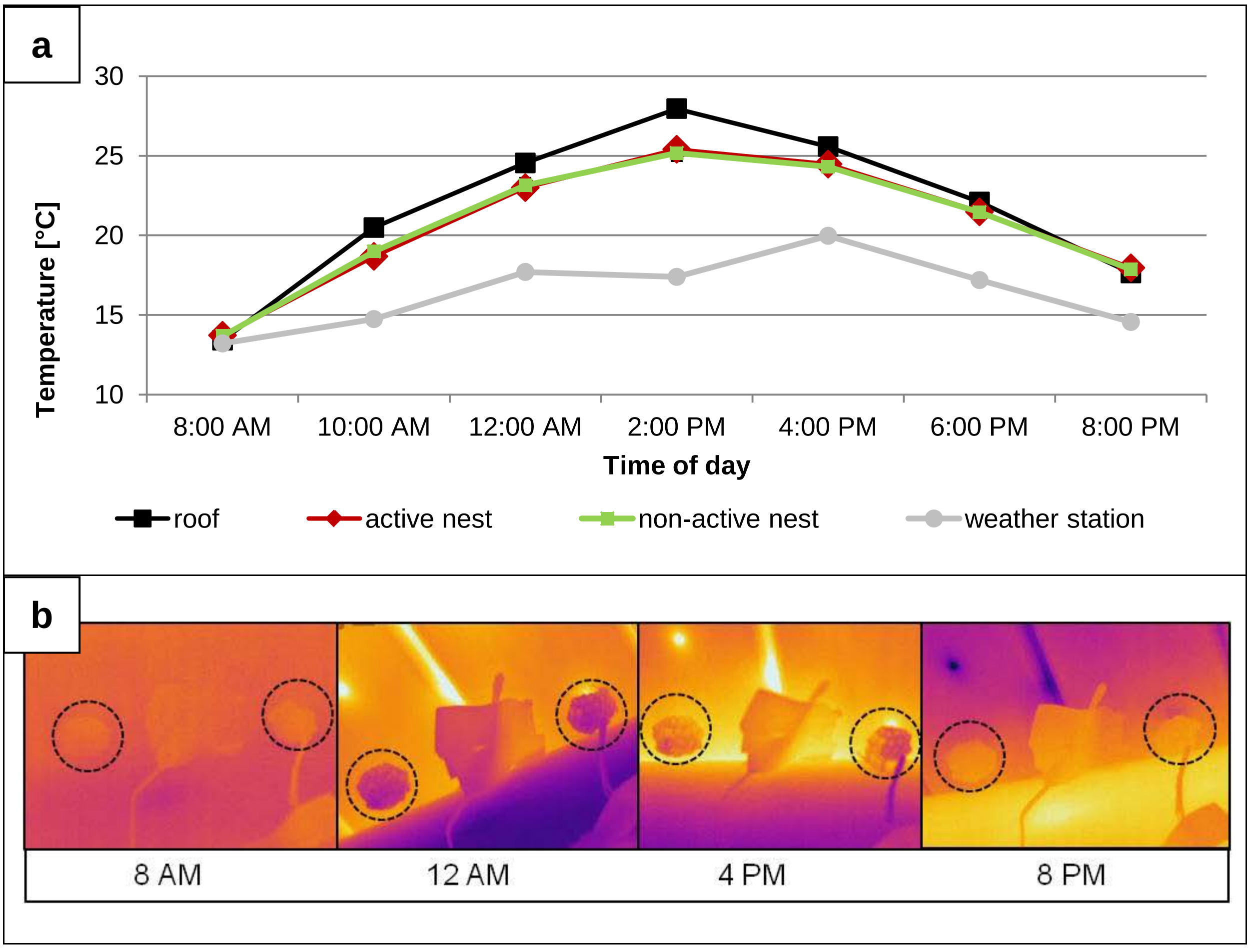


Fig. 27: Comparison of Temperatures of newly founded and old *P. dominula* nests at a maximal outside temperature of 20°C.

(a) Thermographic shots are shown that were recorded at May 9, 2014 (active nest no. 63; site 3; 30 cells; right side; **Table S1**/ non-active nest no. 13; 38 cells; left side; **Table S2**) for the observation of nest temperature. Means ± SD are represented for nest and roof temperatures. Pictures were taken every two hours with an infrared camera for 310 s from 8.00 am to 8.00 am. (b) Thermographic images at selected monitoring points. Yellow/orange = higher temperature, purple/blue = lower temperature. The dotted black circles mark the nests.

c) Thermographic shots over 12h at a maximal outside temperature of 25°C

At a maximal outside temperature of 25°C, the analysis of one representative data set (out of n = 3) showed for the first time an active thermoregulation by the adult wasps. The active and the non-active nest showed different temperatures **(Fig. 28 a)**. The active nest is on average 13.96°C (± 6.72) cooler than the roof between 10.00 am and 6.00 pm, whereas the non-active nest is only 5.75°C (± 1.24) cooler. However, an additional passive thermoregulation is again detectable by considering the non-active nest. At the hottest monitoring points at 2.00 pm and 4.00 pm, the non-active nest displayed temperatures 6.98°C and 7.11°C cooler than the roof, respectively. The temperature differences between the active nest and the roof at the same monitoring points revealed the impressive cooling activity of the wasps (21.24°C and 19.25°C respectively). In this particular case, the active nest reached temperatures above 35°C. This could be an effect of the analysis of the pictures, because four data points were pooled and the edges of the nest are warmer than the brood cells. Again, the nest temperatures of both nests and the roof temperature was nearly the same at 8.00 am and 8.00 pm.

The respective pictures of the thermographic recordings clearly illustrated the differences in temperature between the nests and the roof during the course of the day **(Fig. 28 b)**. The photo at 8.00 am showed that the nests exhibited nearly the same temperature as the roof, while at 12.00 am the active nest appeared visibly cooler than the non-active nest, even if the latter was cooler than the roof. At 8.00 pm the nests reached the roof temperature again. Noteworthy is the observation that the roof can reach a maximal temperature of about 60°C even on a moderate outside temperature of 25°C.

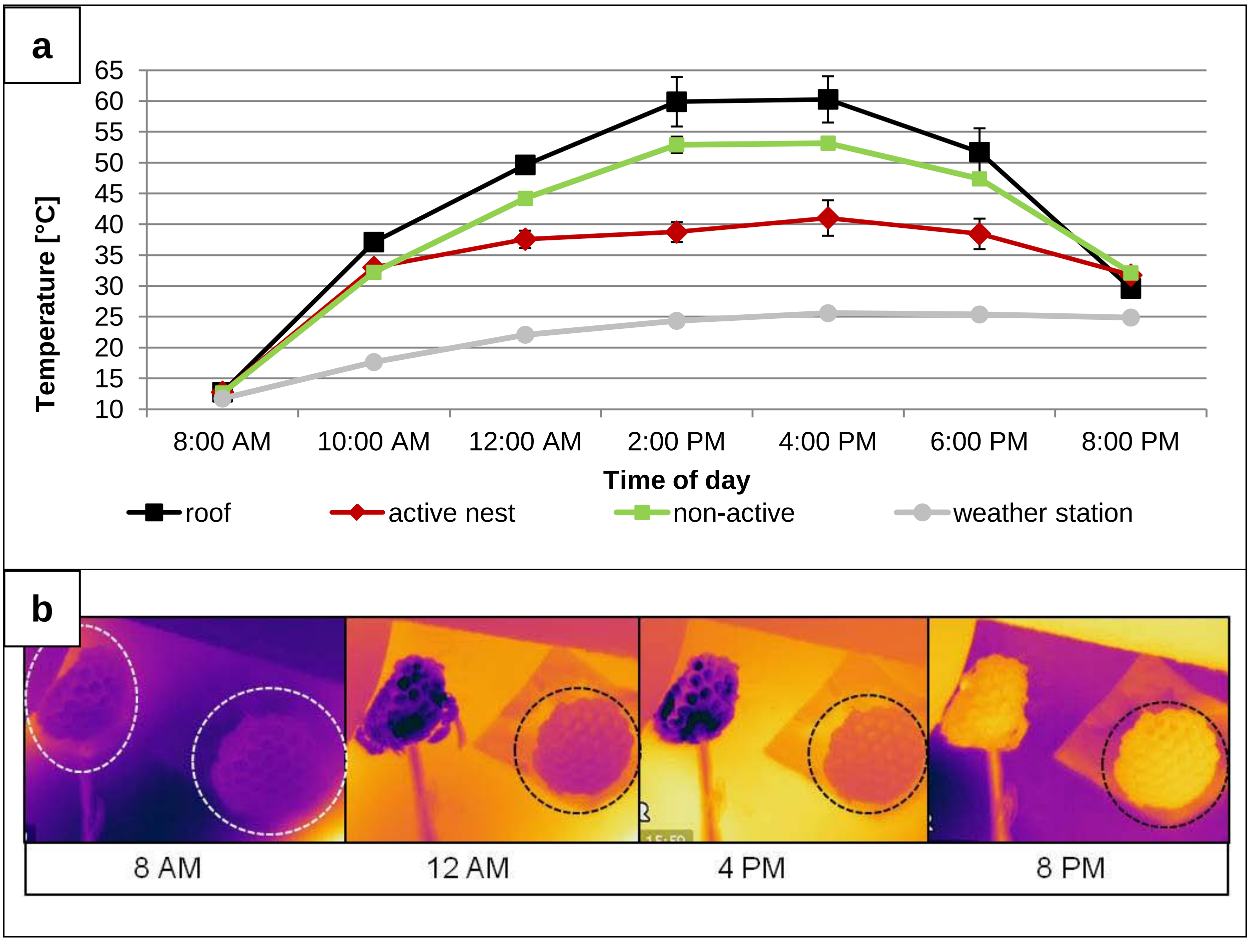


Fig. 28: Comparison of Temperatures of newly founded and old *P. dominula* nests at a maximal outside temperature of 25°C.

(a) Thermographic shots are shown that were recorded at June 6, 2014 (active nest no. 53; site 3; 48 cells; left side; Table S1/ non-active nest no. 5; 55 cells; right side; Table S2) for the observation of nest temperature. Means ± SD are represented for nest and roof temperatures. Pictures were taken every two hours with an infrared camera for 310 s from 8.00 am to 8.00 am. (b) Thermographic images at selected monitoring points. Yellow/orange = higher temperature, purple/blue = lower temperature. The dotted white and black circles mark the nests.

d) Thermographic shots over 12h at a maximal outside temperature of 30°C

As expected, at a maximal outside temperature of 30°C, the analysis of a single data set (out of n = 5) showed an active thermoregulation by the adult wasps, too **(Fig. 29 a)**. The active nest is on average 9.05°C (± 6.68) cooler than the roof between 10.00 am and 6.00 pm, whereas the non-active nest is only 1.91°C (± 2.35) cooler.

An additional passive thermoregulation is again detectable by the non-active nest. At the hottest monitoring point at 4 pm, the non-active nest displayed a temperature 3.54°C cooler than the roof. The temperature differences between the active nest and the roof at the monitoring points 2 pm and 4 pm revealed the impressive cooling activity of the wasps (15.8°C and 14.96°C, respectively).

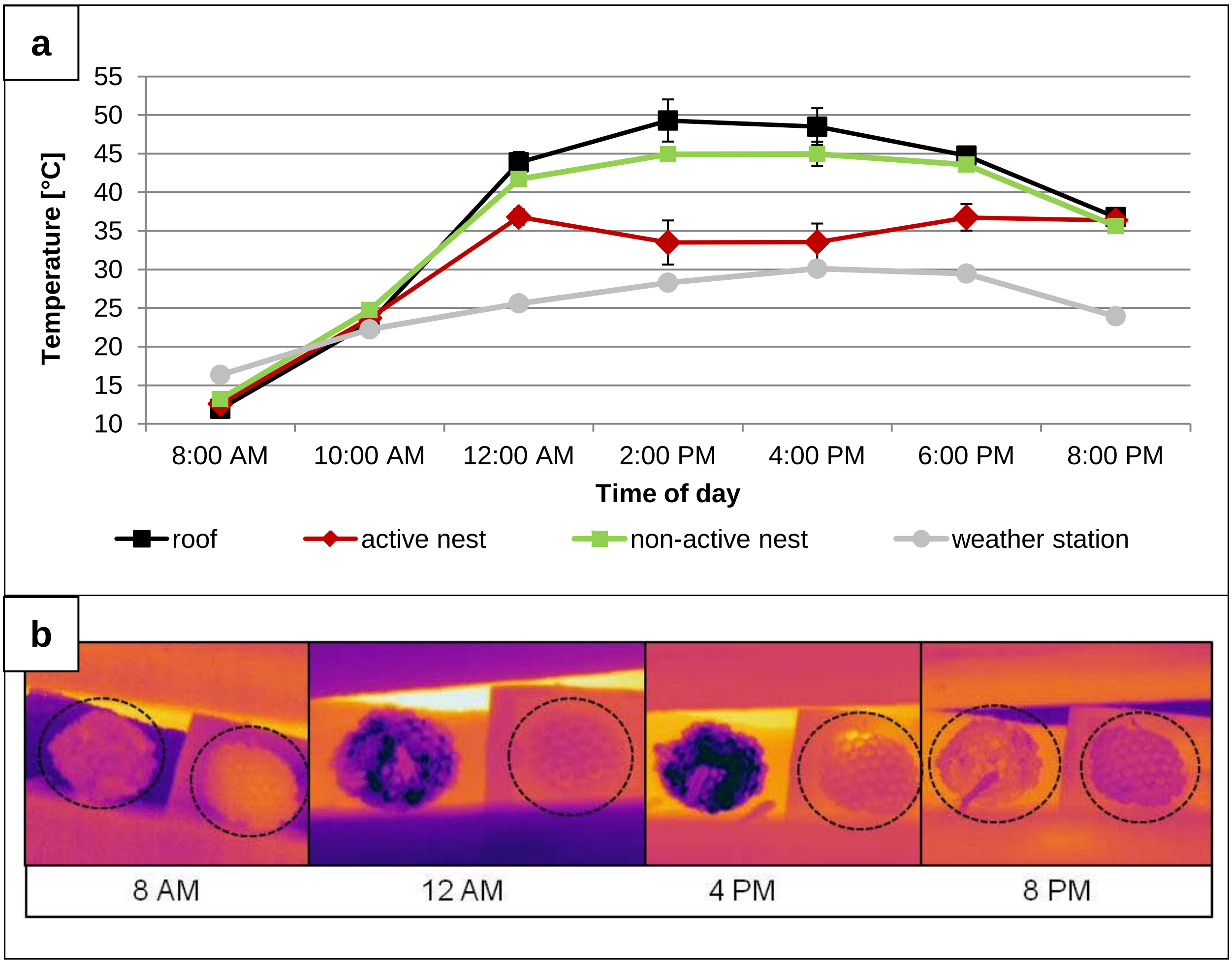


Fig. 29: Comparison of Temperatures of newly founded and old *P. dominula* nests at a maximal outside temperature of 30°C.
(a) Thermographic shots are shown that were recorded at June 7, 2014 (active nest no. 47; site 1; 84 cells; left side; **Table S1**/ non-active nest no. 43; 94 cells; right side; **Table S2**) for the observation of nest temperature. Means ± SD are represented for nest and roof temperatures. Pictures were taken every two hours with an infrared camera for 310 s from 8.00 am to 8.00 am. (b) Thermographic images at selected monitoring points. Yellow/orange = higher temperature, purple/blue = lower temperature. The dotted black circles mark the nests.

In this specific recording (out of n = 5), the temperature of the active nest stayed rather stable around 35°C. This could be explained by taking a look at the respective thermographic pictures **(Fig. 29 b)**. The edges of the nest are as warm as the brood cells. The respective pictures of the thermographic recordings illustrate the differences in temperature between the nests and the roof during the course of the day. The photo at 8.00 am shows that the nests exhibited nearly the same temperature as the roof, while at 12.00 am the active nest appeared visibly cooler than the non-active nest, even if the latter is cooler than the roof. At 8.00 pm, the nests reached the roof temperature again. Despite a higher outside temperature, the roof and hence the nests as well did not achieve such extremely high temperatures as illustrated in **Figure 28,** possibly due to the different location sites.

e) Thermographic shots over 12h at a maximal outside temperature of 35°C

The analysis of a single data set (out of n = 3) at a maximal outside temperature of 35°C **(Fig. 30 a)**, showed nearly the same result as revealed at 30°C **(Fig. 29 a)**. The active nest is on average 11.78°C (± 3.57) cooler than the roof between 10.00 am and 6.00 pm, whereas the non-active nest is only 1.85°C (± 1.34) cooler. The differences between active nest and roof at the monitoring points 2.00 pm and 4.00 pm showed the impressive cooling activity of the wasps (15.16°C and 13.64°C, respectively). Again, the temperature of the active nest stayed stable around 35°C. The respective pictures of the thermographic recordings illustrate the differences in temperature between the nests and the roof during the course of the day **(Fig. 30 b)**.

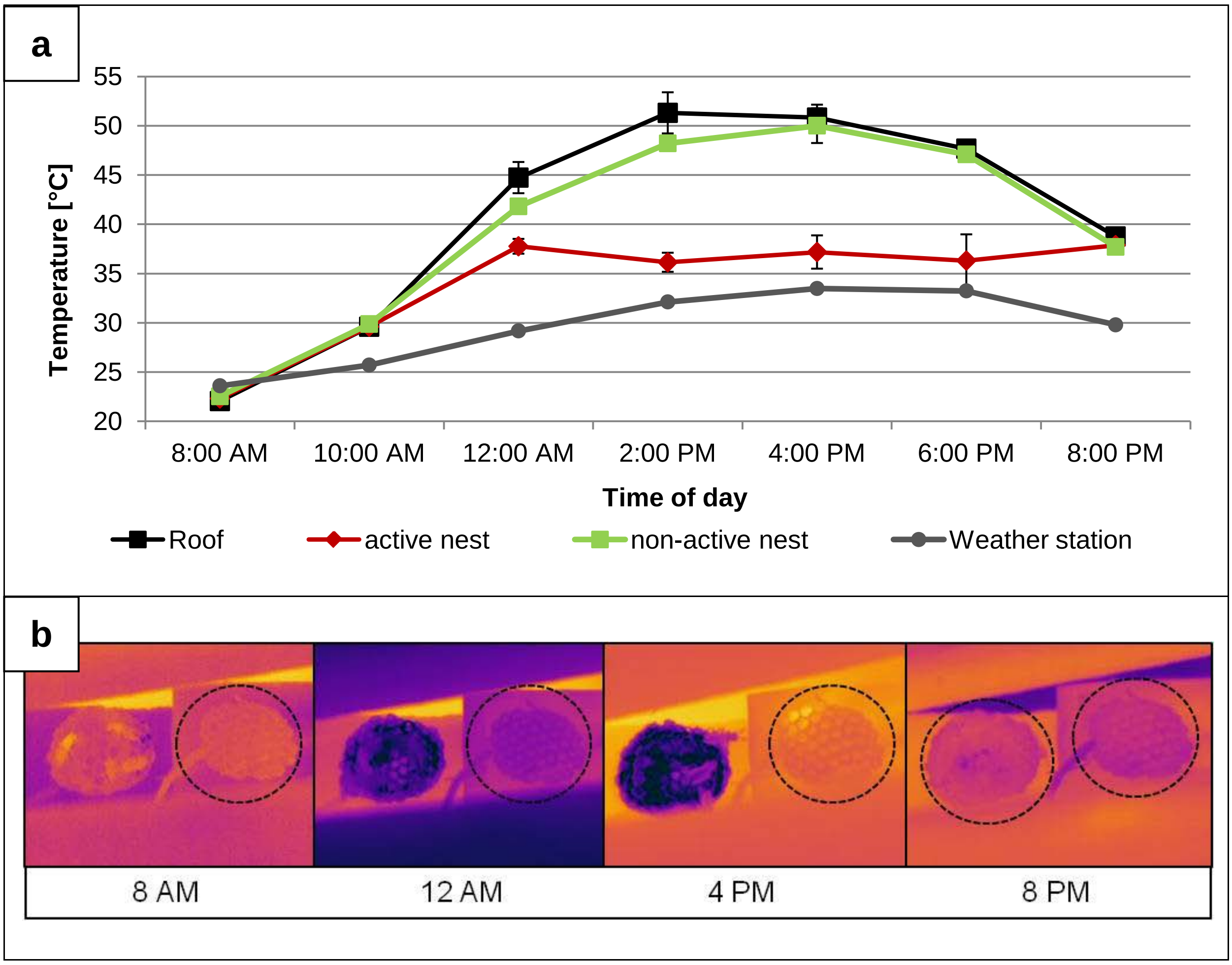


Fig. 30: Comparison of Temperatures of newly founded and old *P. dominula* nests at a maximal outside temperature of 35°C.

(a) Thermographic shots are shown that were recorded at June 10, 2014 (active nest no. 47; site 1; 84 cells; left side; **Table S1**/ non-active nest no. 43; 94 cells; right side; **Table S2**) for the observation of nest temperature. Means ± SD are represented for nest and roof temperatures. Pictures were taken every two hours with an infrared camera for 310 s from 8.00 am to 8.00 am. (b) Thermographic images at selected monitoring points. Yellow/orange = higher temperature, purple/blue = lower temperature. The dotted black circles mark the nests.

4.3.1.4 Adult P. dominula *wasps have a great influence on nest temperature*

As shown in the preceding chapters, adult *P. dominula* wasps can influence the nest temperature by cooling the nest. Over all 21 data sets, it became apparent that the wasps start cooling the nest at maximal outside temperatures above 25°C. These temperatures quite often cause roof temperatures of over 50°C of. This makes an effective cooling of *P. dominula* nests necessary, because the latter are often attached under roof tops (see **Fig. 13**). **Figure 31** shows the mean nest temperature of active and non-active nests dependent on the maximal outside temperature.

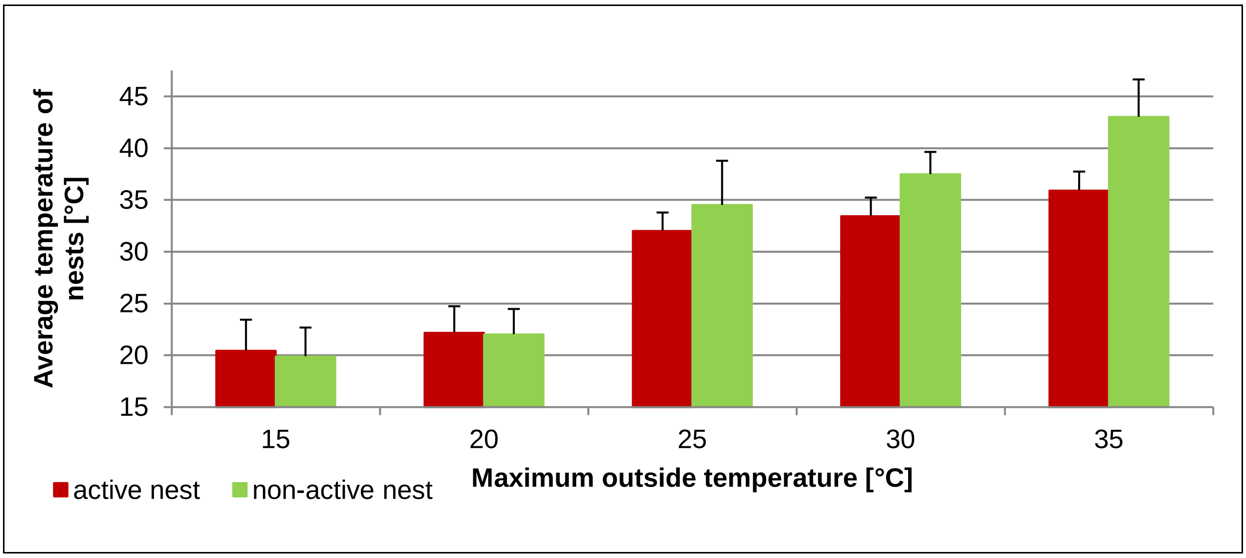


Fig. 31: Comparison of temperatures of active and non-active *P. dominula* nests at different outside temperatures.
Bars represent means ± SD. The cooling effect of the adult wasps on nest temperature is visible above 25°C maximum outside temperature. At 15°C, 20°C and 30°C n = 5; at 25°C and 35°C n = 3.

At cool days (15°C and 20°C), the difference in nest temperature between active and non-active nests is not significant (0.55°C and 0.16°C, respectively). At 25°C, the active nests are on average 2.49°C cooler than the non-active nests. At an outside temperature of 30°C, the active nests are on average 4.07°C colder and at an outside temperature of about 35°C, the active nests are on average 7.11°C cooler than the non-active nests. A two-way ANOVA clearly underlines the significant influence of adult *P. dominula* wasps on the nest temperature (F (1, 32) = 9.582, p = 0.004). The outside temperature obviously had an important effect on the nest temperature, too (F (4, 32) = 88.090, p < 0.0001). In summary, a significant interaction between the tested parameters (activity of the nest=active/non-active and outside temperature) and the nest temperature has been detected (F (4, 32) = 2.81, p = 0.042).

4.3.2 The efficiency of cooling the nests by fanning and water evaporation of adult paper wasps

4.3.2.1 Behaviour of adult wasps differs dependent on the outside temperature

The behaviour of wasps on their nests dependent on the outside temperature was studied while investigating the nest temperatures via infrared camera (see chapter 4.3.1). At every monitoring point (between 8.00 am and 8.00 pm, every two hours for ten minutes), the home coming wasps were observed. Wasps that brought water for cooling were identified with the help of the infrared camera. Cooled cells appeared in dark colours after the wasp had applied water to the cell walls. In addition, wasps that were sitting on the nest and showed a fanning behaviour were also recorded. The data revealed that at outside temperatures of 15°C and 20°C no cooling behaviour took place **(Table 8)**. Water collecting wasps (a total of 50 wasps per 3 nests) were first detected at an outside temperature of 25°C. At the highest outside temperature of 35°C, the number of water collecting wasps increased considerably (i. e., 140 wasps per 3 observed nests). A few wasps were observed during the observation period that showed a fanning behaviour.

Table 8: The behaviour of home coming wasps (12h time interval) differed dependent on the outside temperature.

Temperature [°C]	No. of observed nests	Σ of wasps showing wing fanning[a)]	Σ of wasps applying water[a)]
15°C	5	0	0
20°C	5	2	0
25°C	3	0	50
30°C	5	3	82
35°C	3	2	140

[a)] The nests were recorded between 8.00 am and 8.00 pm every two hours for ten minutes (Σ = 60 min per nest and day; see also 3.4.2.1)

4.3.2.2 Effectiveness of fanning behaviour on nest temperature

Only four cases of cooling events by fanning were recorded with the thermographic camera, during the three years of observation. On the other hand, at least 42 cases of cooling events by water evaporation were detected by using thermal imaging. The analysis of a single case of a cooling event by fanning revealed that this is not an effective thermoregulation in *P. dominula* nests **(Fig. 32 a)**. No considerable decrease in temperature of the cells was detected in the direct ambience of the fanning wasp, after the wasp started to fan. This underlines the statement that fanning cannot be a very important type of active thermoregulation. An illustration by thermographic images was deliberately omitted, since no effect was observed.

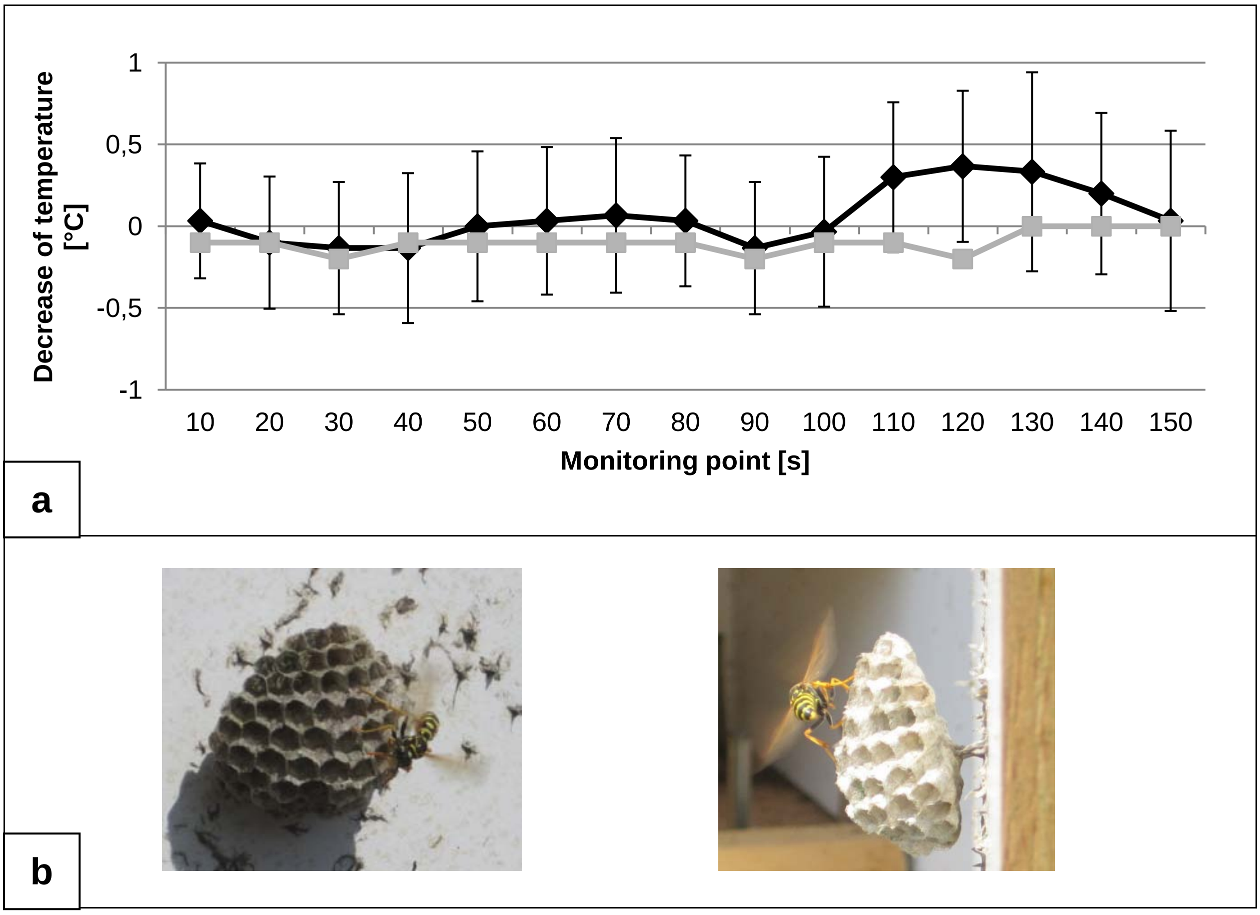


Fig. 32: Cooling event by wing fanning of a single *Polistes dominula* wasp.
(a) Mean differences of temperatures (± SD) of cells (◆, nest no. 62, site 3; July 17, 2014; 117 cells) are shown in the direct ambience of the fanning wasp. Additionally, the differences in thorax temperature of the fanning wasp are represented (■). (b) Two pictures of a fanning wasp; one showing the wasp frontal (left side) and the second picture showing a fanning wasp from behind (right side).

4.3.2.3 Effectiveness of cooling the nest by water collecting wasps

The analysis of a single case of a cooling event - by water application to the cell walls - showed that this is a very effective kind of active thermoregulation in *P. dominula* wasps **(Fig. 33)**. At the start, the relevant open brood cell had a temperature of 36.2°C **(Fig. 33 a)**. Only 10 s after the wasp had applied water to the cell walls, the temperature decreased by 3.8°C **(Fig. 33 c)** and 50 s after the cooling procedure had started, the temperature of the cell had further decreased by 6.1°C **(Fig. 33 d)**. It took about 6 min until the cell nearly reached its initial temperature **(Fig. 33 f)**.

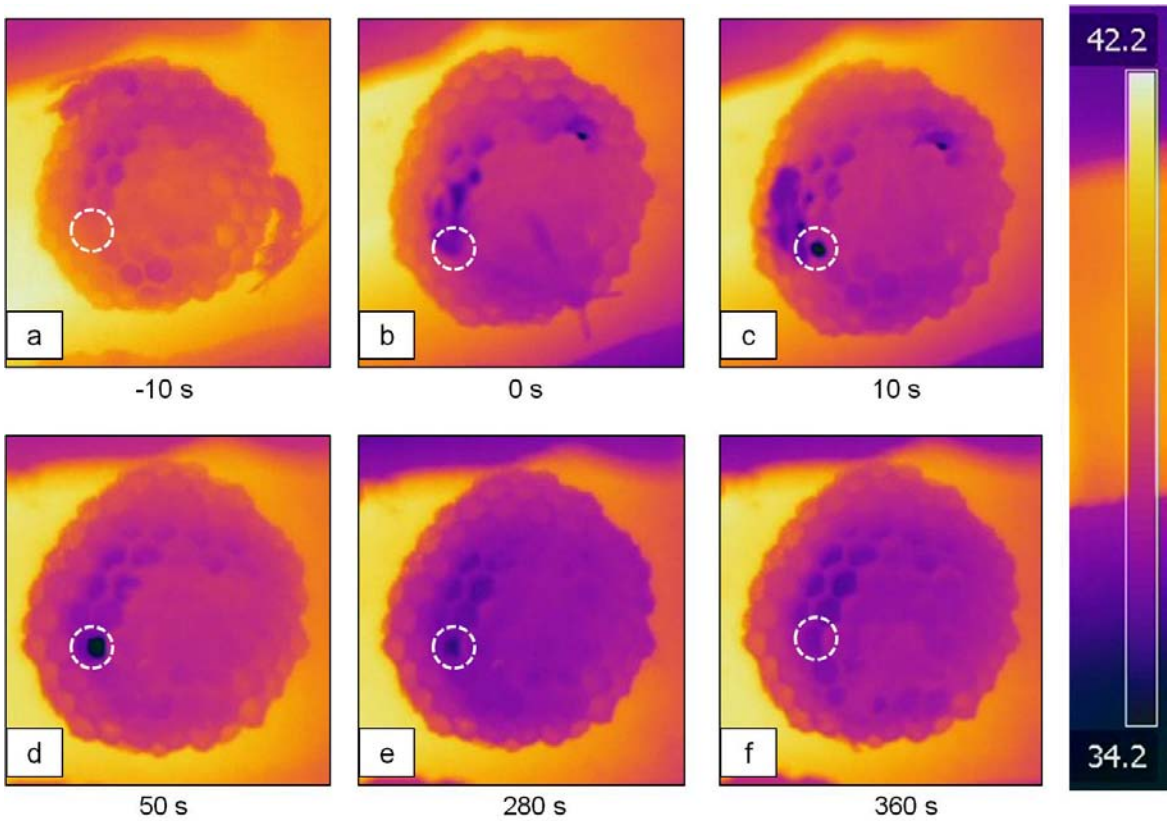


Fig. 33: Course of cooling one single cell in a *P. dominula* nest.
(a) The nest (nest no. 1, site 1; May 30, 2012; 87 cells) with two wasps sitting on it before the cooling event takes place. (b) The wasp (blue shaded in the middle of the nest) applies water to the cell walls. (c) The cell is cooled down (dark blue coloured). (d) The cooling event reaches its peak. (e) The cell warms up again. (f) The cell reaches nearly the starting temperature. Temperature scale of the infrared images is represented at the right side. The white dotted circles mark the respective observed cell.

The analysis of another single case of a cooling event by water evaporation revealed a similar picture **(Fig. 34).** At the start, the relevant open brood cell had a temperature of 37.8°C. Only 10 s after the wasp had applied water to the cell walls, the temperature decreased by 5.2°C. The cooling event reached its minimum 20 s after the application of water and lasted for about 2 min (monitoring points 30 to 150 s). It took 4 min (monitoring point 20 to 240) until the cell nearly reached its initial temperature.

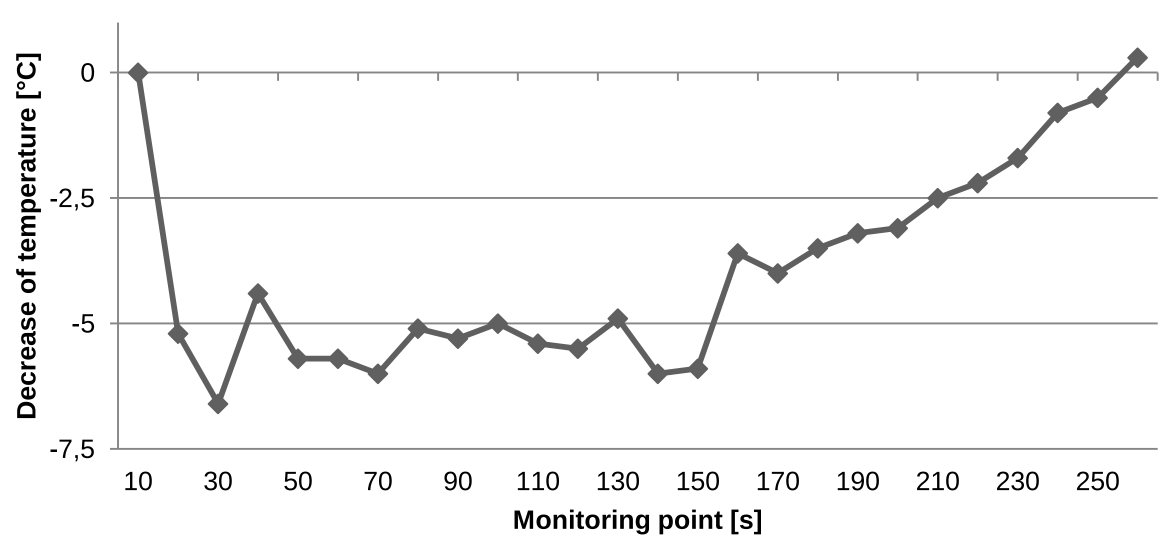


Fig. 34: Course of the cooling effect in a single cell by water evaporating wasps.
A single cooling event is shown that was recorded at June 22, 2012 (nest no. 13, site 3). At that time the nest consisted of 72 cells and harboured six adult wasps. Mean differences of the cell temperature is shown.

Over all 42 analyses of cooling events by water evaporation within a single cell, an average temperature decrease of 7.82°C (± 4.00) was detected. The lowest recorded temperature decrease was 2.4°C and the highest 17.8°C.

Furthermore, in 17 of the 42 analysed cooling events the whole procedure was documented. That means, successive thermographic shots were analysed, from the moment the wasp applied the water to the cell wall until the cell reached again the starting temperature. Therefore it was possible to investigate how long a cell was in fact cooled down by water. Cooling of a single cell by water evaporation lasted at least 240 s, but on average it took 392.94 s (± 153.69) until the cell reached the basic temperature. Maximally, this cooling event persisted for 630 s. These observations indicate how effective the active thermoregulation of the nest is achieved by cooling the cells through application of water to the cell wall.

4.3.3 The influence of nest size on the nest temperature

As described in 4.3.1, a passive thermoregulation takes place in *P. dominula* nests revealed by comparing active and non-active nests at higher outside (and roof) temperatures. At first glance, the size of the nests could play a significant role in the passive thermoregulation. In order to investigate this assumption, a specially devised experiment was carried out. For this, nests of different sizes (size cohorts: 31-60 (size 1); 61-90 (size 2); 91-120 (size 3); 121-150 cells (size 4); 3 nests per size; different orientation) were hung under the roof of a natural *P. dominula* location and investigated by using the thermographic camera. The recordings were made for 24 h at days with a maximal outside temperature of 10°C (April 14, 2014), 15°C (April 8, 2014), 20°C (April 22, 2014), 25°C (June 6, 2014), 30°C (May 21, 2014) and 35°C (June 9, 2014). Data sets of 15°C, 25°C and 35°C are shown in the following chapters.

4.3.3.1 Thermographic recordings of different sized non-active P. dominula *nests*

a) Thermographic shots at a maximal outside temperature of 15°C

Obvious differences between the roof temperatures and the temperatures of nests of different sizes could be detected even on a cool day with a maximal outside temperature of 15°C **(Fig. 36)**. Between 10.00 am and 4.00 pm, the nests were colder than the roof. During the night, between 8.00 pm and 8.00 am, the nests stay warmer than the roof. On average the nests of group 1 were 2.39°C (± 2.22) colder during the day and 1.71°C (± 0.72) warmer during the night. Nests of size 2 were on average 2.52°C (± 2.41) cooler and 1.74°C (± 0.68) warmer, respectively. During the day, the nests of size 3 showed a 2.66°C (± 2.51) colder temperature as the roof. During the night, the same group was on average 1.64°C (± 0.60) warmer than the roof. The same effect was detected for nests of group “size 4”. The nests were on average 3.57°C (± 3.36) cooler than the roof, whereas during the night, they showed a temperature 2.02°C (± 0.71) above the roof temperature **(Fig. 35 a)**. It seemed that the biggest nests stayed colder during the day than the other groups. But by taking a closer look at the data points of nest temperatures, it became apparent that no great difference exists. Between nests of size 1 (smallest nests in this experiment) and nests of size 4 (biggest nests in this experiment), differences of 1.18°C during the day and 0.31°C during the night could be detected, but the standard deviations are almost as high as the mean values.

The respective pictures of the thermographic recordings illustrate the differences of the temperatures between the nests and the roof during the course of the day, but they also clearly underline the similarity of the temperatures of nests of all four different sizes **(Fig. 35 b)**.

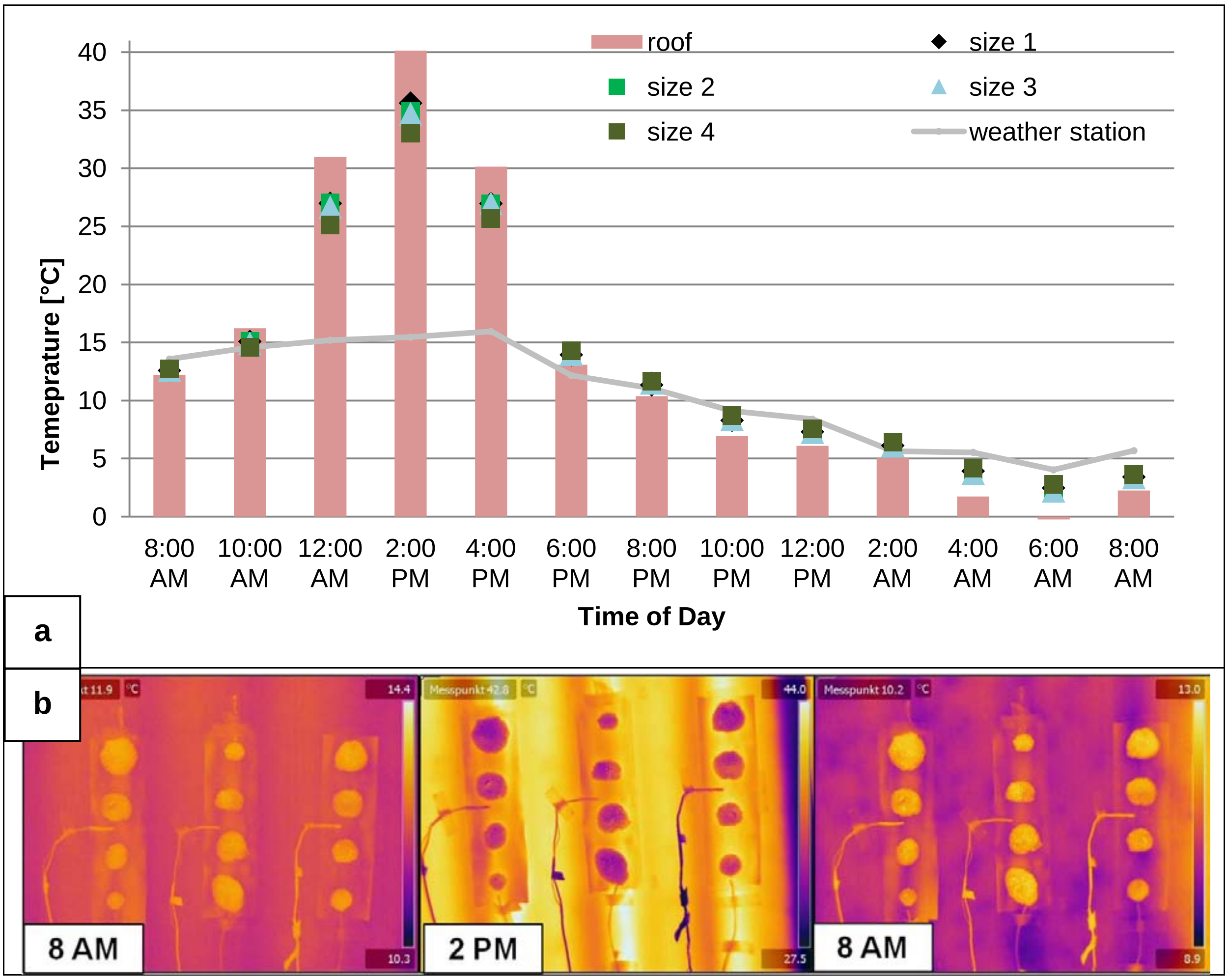


Fig. 35: Comparison of the temperatures of different sized non-active *P. dominula* nests at a maximal outside temperature of 15°C.

(a) The analysis of thermographic shots is shown which were recorded at April 8, 2014 at site 3. Bars represent the average roof temperature; whereas the line shows the weather station data and the dots indicate the mean values of temperatures of nests of four different sizes (size 1 = 31-60 cells; size 2 = 61-90 cells; size 3 = 91-120 cells; size 4 = 121-150 cells). Pictures were taken every two hours with an infrared camera for 310 s from 8.00 am to the next day 8.00 am.

(b) The respective thermographic images at selected monitoring points: 8.00 am, 2.00 pm and 8.00 pm. Yellow/orange = higher temperature, purple/blue = lower temperature.

b) Thermographic shots at a maximal outside temperature of 25°C

On a day of a moderate maximum outside temperature of 25°C, between 10.00 am and 6.00 pm, the nests were colder than the roof. During the night, between 8.00 pm and 6.00 am, the nests stay warmer than the roof **(Fig. 36)**. On average, the nests of size 1 were 4.23°C (± 0.77) colder during the day and 2.25°C (± 0.33) warmer during the night. Nests of size 2 were on average 5.10°C (± 0.95) cooler and 2.21°C (± 0.40) warmer, respectively. During the day, the nests of size 3 showed a 5.36°C (± 1.07) colder temperature as the roof. During the night, the same group were on average 2.11°C (± 0.37) warmer than the roof. The same effect was detected for nests of group "size 4". The nests were on average 6.6°C (± 1.41) cooler than the roof, whereas during the night, they showed a temperature 2.64°C (± 0.46) above the roof temperature **(Fig. 36 a)**.

During the day, the biggest nests stay colder as the other groups. By taking a closer look at the data points of nest temperatures, it became apparent that differences exist. Between nests of size 1 and nests of size 4, there is a difference of 2.37°C during the day. During the night a difference of only 0.39°C was detected. This result gives the impression that bigger nests can stay cooler for a longer time as the small nests can.

The respective pictures of the thermographic recordings illustrate the differences of the temperatures between the nests and the roof during the course of the day, but they also clearly underline the similarity of the temperatures of nests of all four different sizes **(Fig. 36 b)**. In thermographic images, a difference in temperature of "only" about 2°C must not be forcibly obvious, especially not when mean values of every nests are calculated.

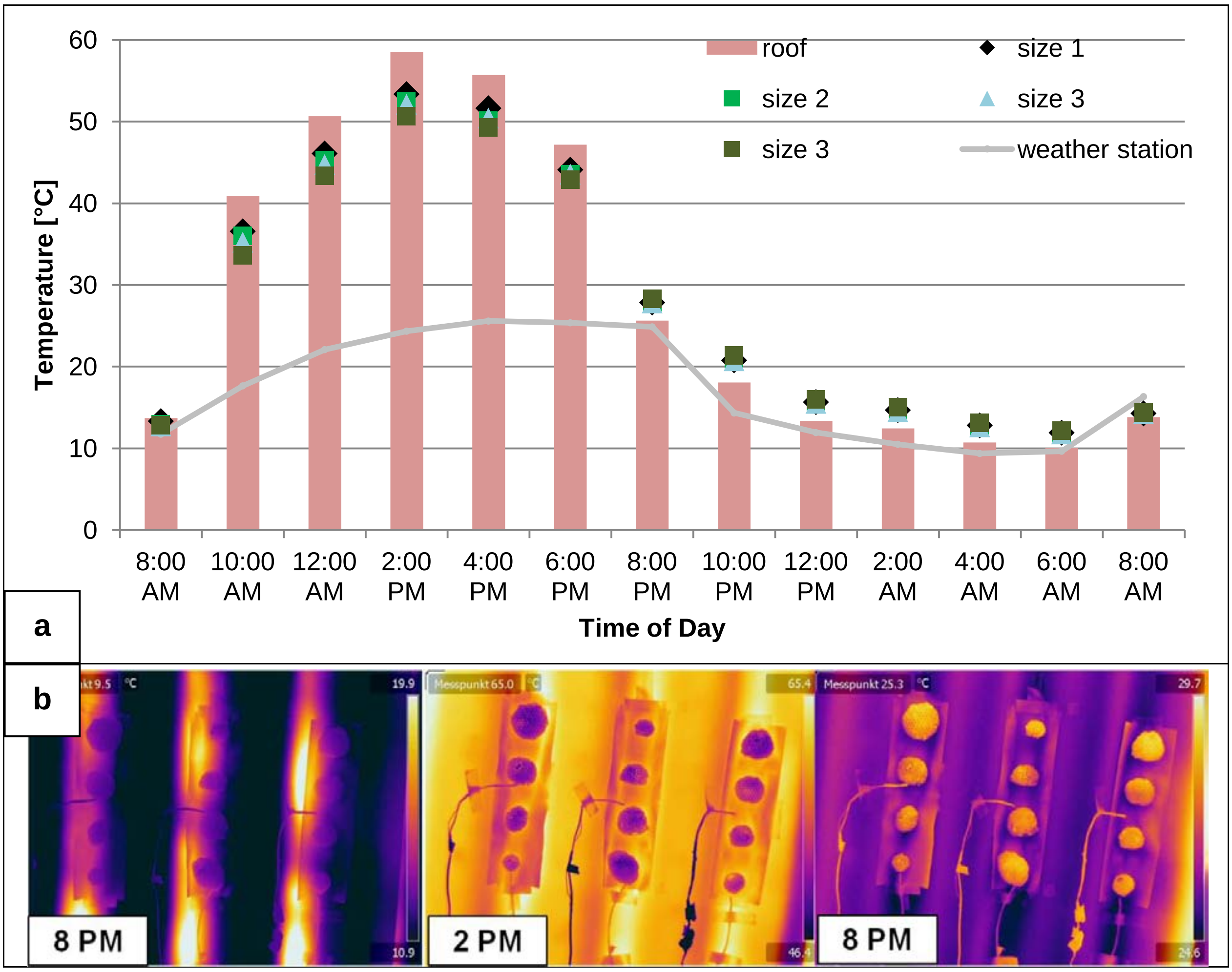


Fig. 36: Comparison of the temperatures of different sized non-active *P. dominula* nests at a maximal outside temperature of 25°C.

(a) The analysis of thermographic shots is shown which were recorded at June 6, 2014 at site 3. Bars represent the average roof temperature; whereas the line shows the weather station data and the dots indicate the mean values of temperatures of nests of four different sizes (size 1 = 31-60 cells; size 2 = 61-90 cells; size 3 = 91-120 cells; size 4 = 121-150 cells). Pictures were taken every two hours with an infrared camera for 310 s from 8.00 am to the next day 8.00 am.

(b) The respective thermographic images at selected monitoring points: 8.00 am, 2.00 pm and 8.00 pm. Yellow/orange = higher temperature, purple/blue = lower temperature.

c) Thermographic shots at a maximal outside temperature of 35°C

On a hot day with a maximum outside temperature of 30°C, the nests were also colder than the roof between 10.00 am and 6.00 pm. Between 8.00 pm and 6.00 am, the nests stay warmer than the roof **(Fig. 37)**. On average the nests of size 1 were 4.20°C (± 1.32) colder during the day and 1.99°C (± 0.36) warmer during the night. Nests of size 2 were on average 4.88°C (± 1.43) cooler and 1.83°C (± 0.30) warmer, respectively. During the day, the nests of size 3 showed a 4.88°C (± 1.51) colder temperature as the roof. During the night, the same group were on average 1.78°C (± 0.24) warmer than the roof. The same effect was detected for nests of group "size 4". The nests were on average 6.30°C (± 1.87) cooler than the roof, whereas during the night, they showed a temperature 2.34°C (± 0.34) above the roof temperature **(Fig. 37 a)**.

During the day, the biggest nests stayed colder as the other groups. By taking a closer look at the data points of nest temperatures, it became apparent that differences exist, especially during the day. Between nests of size 1 and nests of size 4, during the day there is a difference of 2.1°C. During the night a difference of only 0.35°C was detected. This result gives again the impression that bigger nests can stay cooler for a longer time as the small nests can.

The respective pictures of the thermographic recordings illustrate the differences of the temperatures between the nests and the roof during the course of the day, but they also clearly underline the similarity of the temperatures of nests of all four different sizes **(Fig. 37 b)**. Again, the difference in temperature is not visible in the pictures.

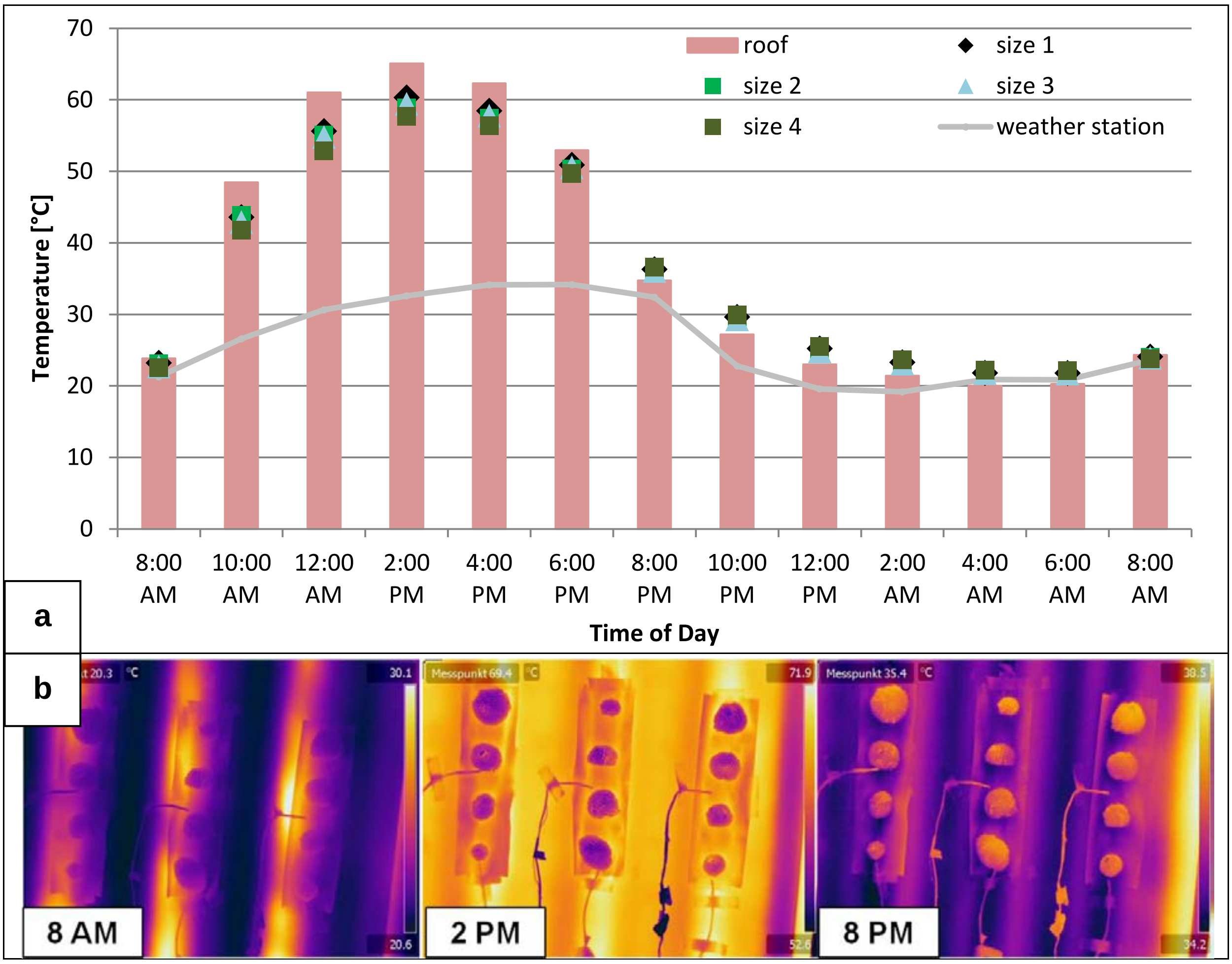


Fig. 37: Comparison of the temperatures of different sized non-active *P. dominula* nests at a maximal outside temperature of 35°C.

(a) The analysis of thermographic shots is shown which were recorded at June 9, 2014 at site 3. Bars represent the average roof temperature; whereas the line shows the weather station data and the dots indicate the mean values of temperatures of nests of four different sizes (size 1 = 31-60 cells; size 2 = 61-90 cells; size 3 = 91-120 cells; size 4 = 121-150 cells). Pictures were taken every two hours with an infrared camera for 310 s from 8.00 am to the next day 8.00 am.

(b) The respective thermographic images at selected monitoring points: 8.00 am, 2.00 pm and 8.00 pm. Yellow/orange = higher temperature, purple/blue = lower temperature.

4.3.3.2 The size of P. dominula *nests plays no important role in passive thermoregulation*

As shown in the former paragraphs (4.3.1 and 4.3.2.1), the nest temperature in non-active nests is influenced by passive thermoregulation. Over all data sets, it became apparent that the nests stay cooler than the roof (influenced by the outside temperature) during the day and that they stay warmer than the roof during the night. Hot outside temperatures quite often create roof temperatures above 50°C. **Figure 38** shows the mean nest temperature of non-active nests of different sizes dependent on the maximal outside temperature. The data were split in recordings made by daytime (8.00 am to 8.00 pm; **Fig. 38 a**) or night time (8.00 pm to 8.00 am; **Fig. 38 b**), because of the thermal behaviour of the nests.

Taking all the data into account, the same picture is always reflected. As expected, an increase of the outside temperature caused increased nest temperatures. When a closer look is taken to the different nest size groups with respect to increasing outside temperatures, it became apparent that during the day the biggest nests (group "size 4": 121-150 cells) always seemed to be a bit cooler than the other three groups of nests. Furthermore, the groups "size 2" (61-90 cells) and "size 3" (91-120 cells) always showed nearly the same temperatures whereas the smallest nests (group "size 1: 31-60 cells) seemed to be a little warmer than the other three groups of nests, especially at higher temperatures (above 25°C).During the night, a similar picture emerged. The biggest nests seemed to be a bit warmer than the other groups, whereas group "size 1" displayed temperatures like the groups "size 2" and "size 3".

However, a significant interaction (two-way ANOVA) between the tested parameters (nest size = size 1/ size 2/ size 3/ size 4 and outside temperature) and nest temperature was not detectable, neither for the day (F (15, 48) = 1,412, p = 0,1803), nor for the night (F (15, 48) = 0,1578; p = 0,9998).

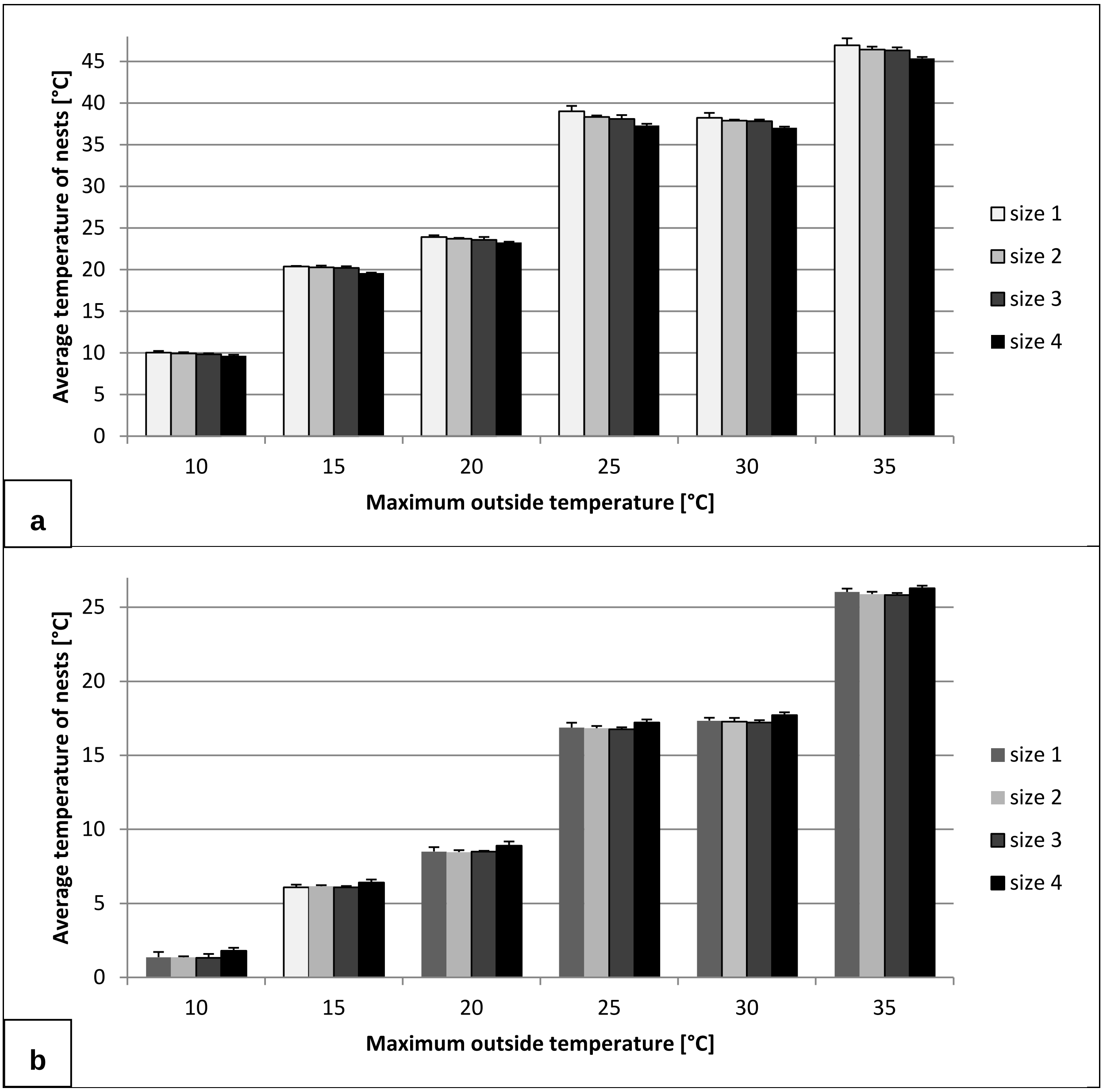


Fig. 38: Comparison of temperatures of different sized non-active *P. dominula* nests at different outside temperatures.

Bars represent means ± SD. At all measured maximum outside temperatures of the observed day (10 – 35°C in 5°C steps), 3 nests per size (n = 12) were recorded at site 3. (a) Mean values are shown for daytime (8.00 am to 8.00 pm). (b) Means are shown for the night time (8.00 pm to 8.00 am); consider the different y-axes.

4.3.4 The influence of the nesting site on the nest temperature

4.3.4.1 Different nesting sites implies different temperature conditions

Nesting at sheltered nesting sites could be a big advantage by protecting against intruders as well as against extreme temperatures. *P. dominula* wasps particularly require warm sheltered nesting sites because of their unenveloped nests. In this study, *P. dominula* nests were found exclusively in nesting sites closely located to human constructions. The temperature of different sites was investigated by using temperature data loggers which recorded the ambient temperature of nests every 10 minutes. In the following study, site no. 1 (barn with a roof made up of tiles), site no. 2 (metal tube at a vineyard) and site no. 3 (barn with a roof made up of corrugated eternit panels) were compared in terms of their temperature at a cool **(Fig. 39 a)** and a hot day, respectively **(Fig. 39 b)**.

At the cool day, it became apparent that the temperature of the metal tubes (site 2) rose rapidly in the morning after sunrise. Between 7:20 am and 9:10 am, the site was on average 9.49°C warmer than site 1 and 6.31°C warmer than site 3. During daytime, site 3 (corrugated eternit panels) was much warmer than the other two sites. Between 10:00 am and 6:30 pm, this site was on average 5.86°C warmer than site 1 and 9.71°C warmer than site 2. The different development of temperature is also noteworthy. Site 1 reached the hottest temperature (34.2°C) already at 1:00 pm, whereas site 2 achieved this point (30.5°C) not until the late afternoon (4:30 pm). Site two reacted completely different and obtained the warmest temperature at 2.00 pm (41.9°C; **Fig. 39 a)**. At the hot day, a similar picture emerged. Site 2 is the coolest site of all studied sites. During the day it is 5.04°C cooler than site 1 and 6.90°C cooler than site 3. The hottest temperatures of site 1 and site 3 are similar, but not the time frame. Site 1 obtained the hottest temperature (51.2°C) at 2:30 pm, whereas site 3 achieved this point already 40 minutes before (1:40 pm; 52.8°C). The hottest temperature of site 2 was reached in the evening (5:20 pm; 43.5°C; **Fig. 39 b)**.

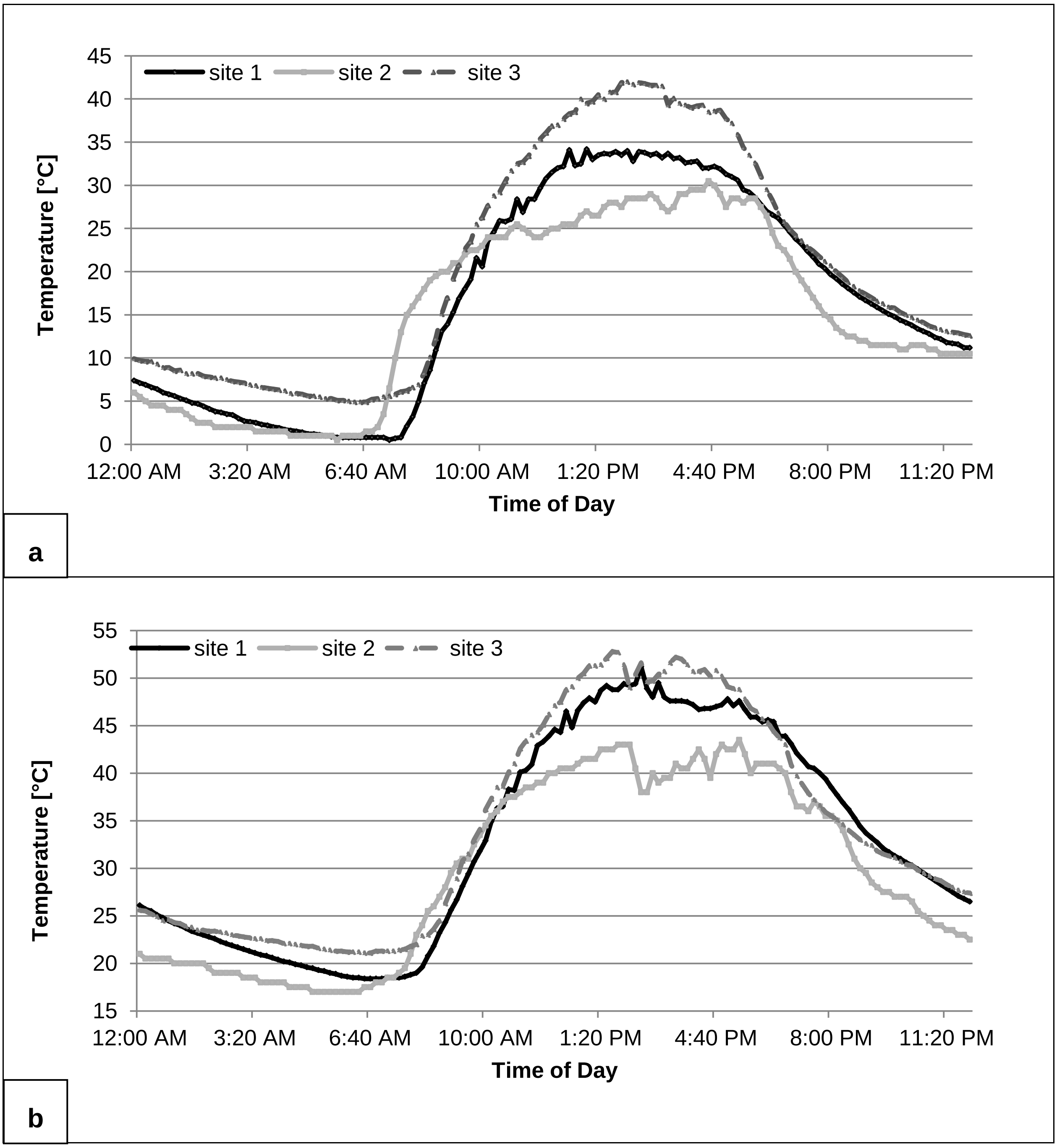


Fig. 39: Ambient temperatures of nests at different sites during the course of day.
Data were recorded with temperature data loggers at site no. 1 (barn with a roof made up of tiles), site no. 2 (metal tube at a vineyard), site no. 3 (barn with a roof made up of corrugated eternit panels; see **Fig. 13**). The data loggers were set in a way that they measured the temperature every 10 minutes. Consider different y-axes. (a) Data are shown that were recorded at May 14, 2012, which was a cool day with a maximum outside temperature of about 17°C. (b) Data show a hot day (July 27, 2012), with a maximum outside temperature of about 35°C.

4.3.4.2 Different locations of nests at the same nesting site imply different temperature conditions

As was revealed in the previous paragraph, different nesting sites can lead to different temperature conditions. Mostly, more than one nest was found at one nesting site during the same year of observation. Due to this fact, it was possible to investigate the ambient temperatures of more than one nest. **Figure 40** shows two of these cases during the first year of observation in 2012.

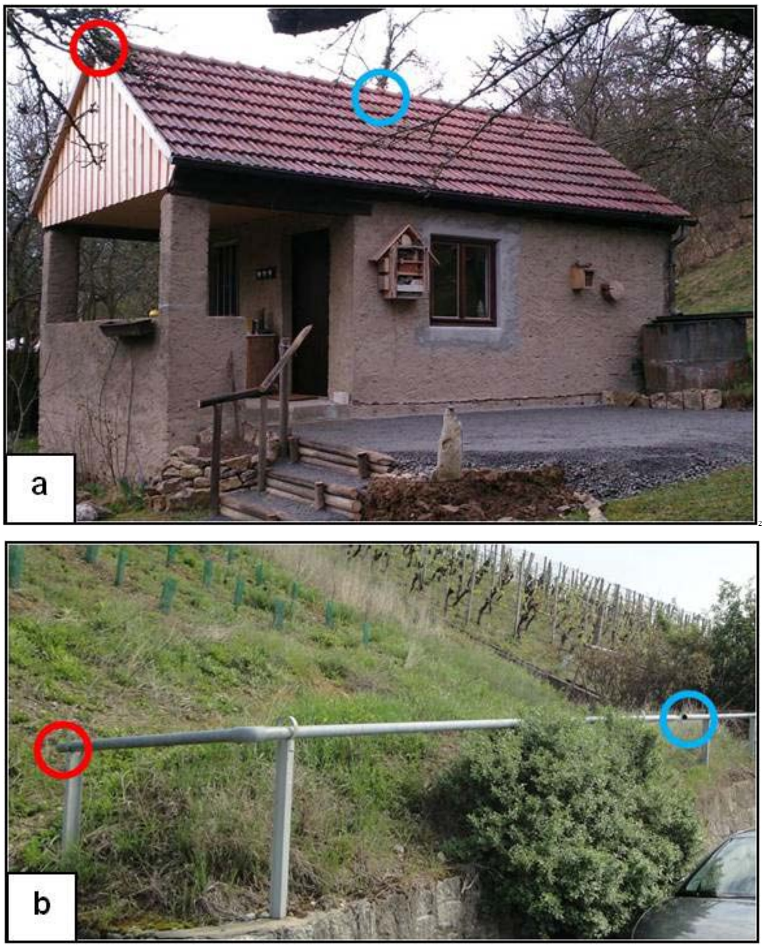


Fig. 40: Localisation of two different nests at the same sites.
(a) Site 1 with a roof made up of tiles. The red circle marks the point where nest 2 was located and the blue circle indicates where nest 3 was found during the observation year of 2012. (b) Site 2, the metal tubes at a vineyard. The red circle signed the place where nest 6 was found and the blue circle shows where nest 7 was located during the year of 2012.

During the cool day, it became apparent that site 1 showed differences within the nesting site. A closer look into the data revealed that both observed nests had nearly the same ambient temperature development, but the ambience of nest 2 showed a delay in .time of about 40 min **(Fig. 41 a)**. Nevertheless, the air surrounding nest no. 3 was slightly warmer (average temperature 17.19°C) than that of nest 2 (average temperature 16.81°C) during the day. The temperature surrounding nest no. 2 reached the hottest point (34.2°C) at 12:30 am, whereas the ambient temperature of nest no. 3 obtained this point (35.8°C) about 40 min later at 1:10 pm.

The temperature of the metal tubes (site 2) also increased differently in the direct ambience of the two nests. Between 6:40 am and 11:20 am, the ambient temperatures of nest 6 were on average 4.69°C warmer than that of nest 7. During the rest of the day and during the night, both ambient temperatures were nearly the same **(Fig. 41 b)**. The ambient temperature of nest 7 reached the hottest temperature not until the late afternoon (4:50 pm; 30.5°C), whereas the air surrounding nest 6 achieved this point already at 1:30 pm (28.5°C).

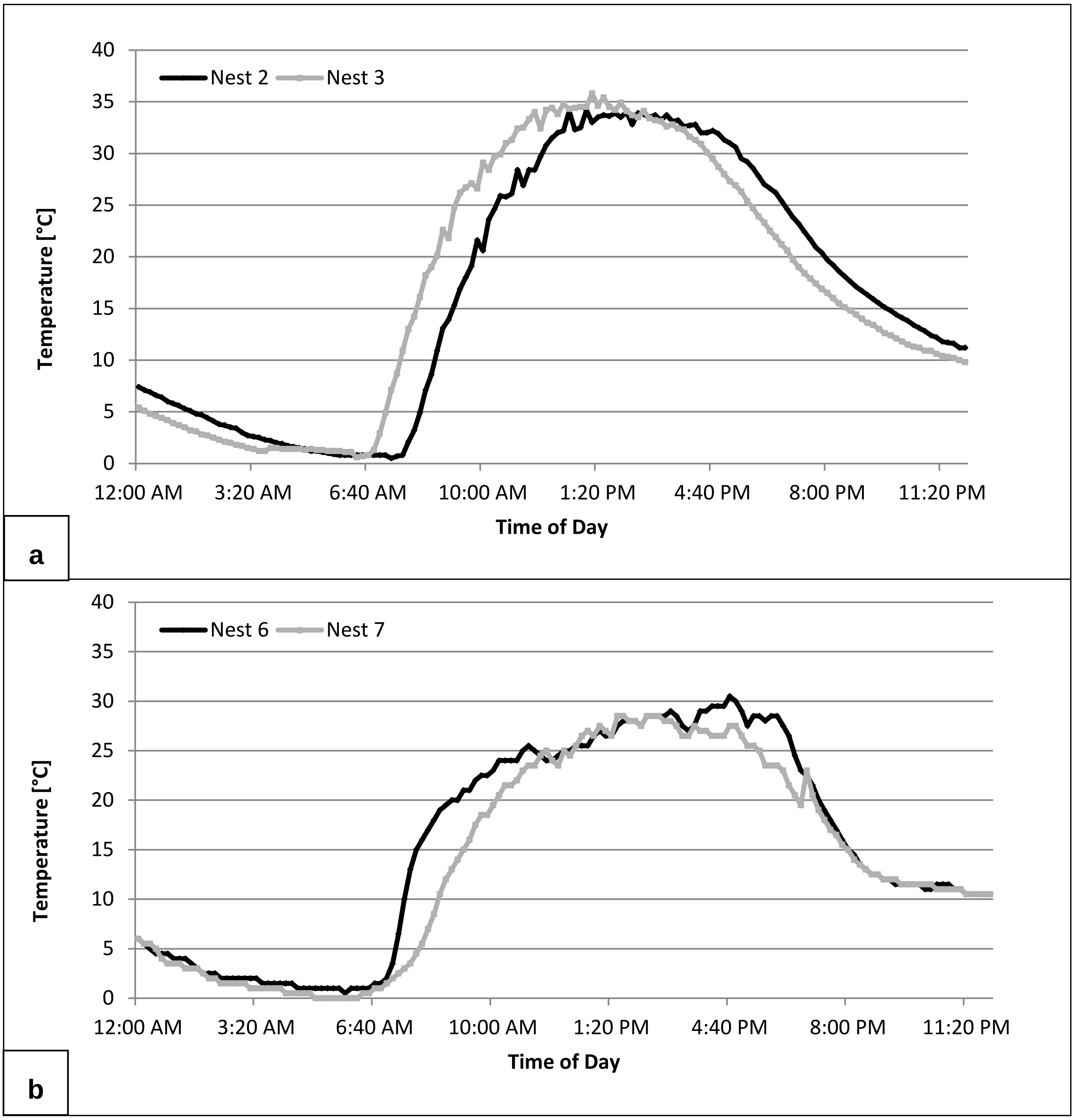


Fig. 41: Ambient temperatures of different nests at the same sites during the course of a cool day.

Data were recorded with temperature data loggers at site no. 2 (metal tube at a vineyard) and site no. 1 (barn with a roof made up of tiles). The data loggers were set so that they measured the temperature every 10 minutes. Data are shown that were recorded at May 14, 2012, which was a cool day with a maximum outside temperature of about 17°C. (a) The temperature is represented of the direct ambience of nest 2 and nest 3 at site 1 **(Table S1)** nest during the course of the day. (b) The temperature is indicated of the direct ambience of nest 6 and nest 7 at site 2 **(Table S1)** during the course of the day.

During the hot day, it became apparent that the temperature at site 1 show again differences within the nesting site. Both observed nests had nearly the same ambient temperature development, but the air temperature surrounding nest 2 showed a delay in time (**Fig. 42 a)**. During the night, between 12:00 pm and 7:30 am, the ambience of nest 2 was slightly warmer than that of nest no. 3 (average temperature 20.72°C and 18.99°C, respectively). In the following, between 7:30 am and 2:30 pm, the temperature surrounding nest no. 3 was on average 4.75°C warmer than that of nest 2. After 4:40 pm, the ambient temperature of nest 2 was warmer than that of nest 3 (average temperature 37.62°C and 35.16°C, respectively).

The temperature of the metal tubes (site 2) also increased in nearly the same way. Between 6:40 am and 11:30 am, a very strong rise of air temperature was observable **(Fig. 42 b)**. The ambient temperature of nest 7 reached the hottest temperature at 1:50 pm (45°C), whereas the air surrounding nest 6 achieved this point not until the evening (5:20 pm; 43.5°C). Noteworthy is that between 11:30 am and 3:40 pm, the ambience of nest 7 is slightly warmer, but after 3:40 pm, the air temperature of nest 6 is slightly warmer than that of nest no. 7.

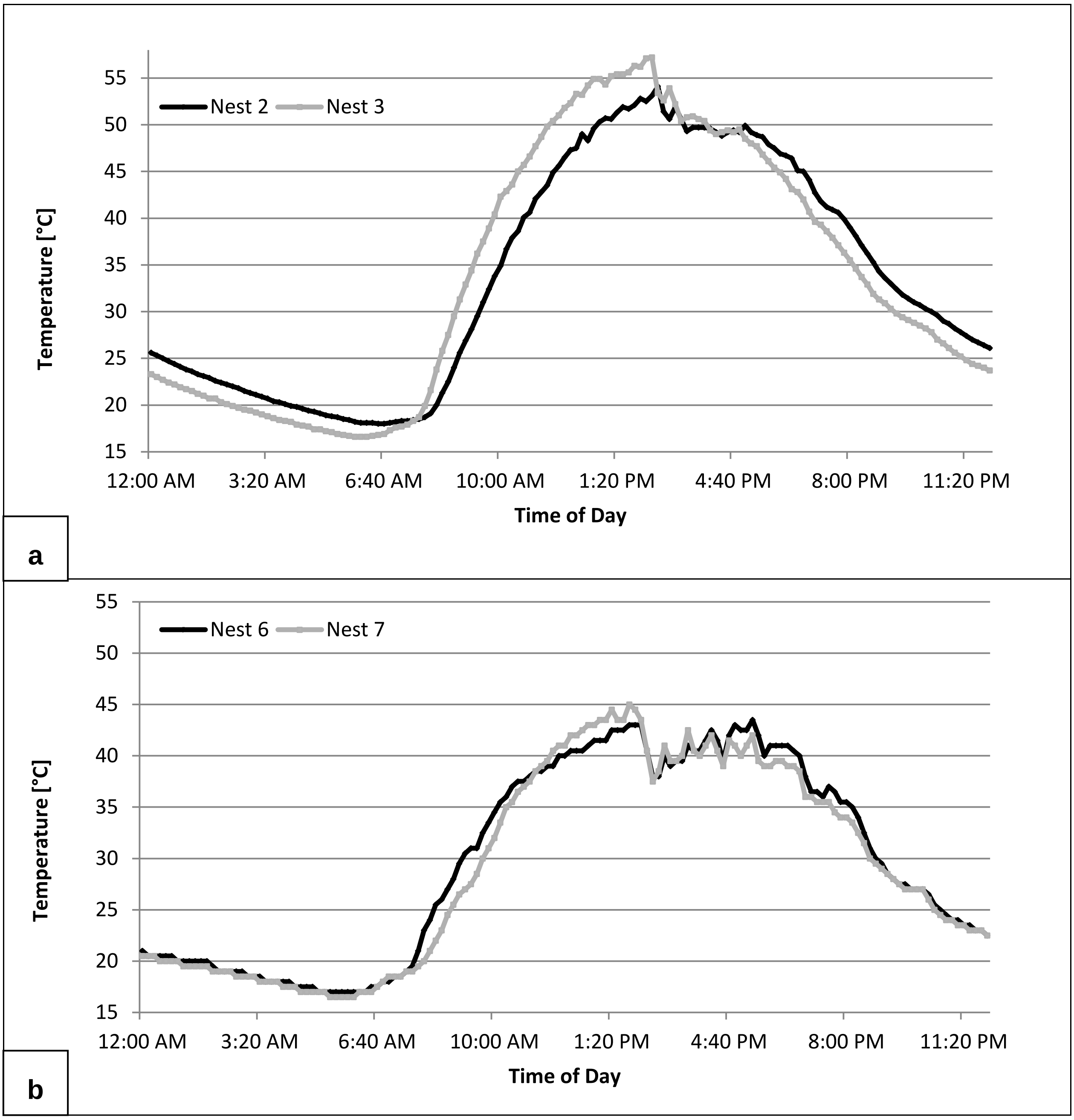


Fig. 42: Ambient temperatures of different nests at the same sites during the course of a hot day.

Data were recorded with temperature data loggers at site no. 2 (metal tube at a vineyard) and site no. 1 (barn with a roof made up of tiles). The data loggers were set so that they measured the temperature every 10 minutes. Data are shown that were recorded at July 27, 2012, which was a hot day with a maximum outside temperature of about 35°C. (a) The temperature is represented of the direct ambience of nest 2 and nest 3 at site 1 **(Table S1)** nest during the course of the day. (b) The temperature is indicated of the direct ambience of nest 6 and nest 7 at site 2 **(Table S1)** during the course of the day.

5. DISCUSSION

5.1 THE NESTING BEHAVIOUR OF *POLISTES DOMINULA* REVEALS A REMARKABLE FLEXIBILITY

Within the last 35 years, *P. dominula* has attracted special attention because of the worldwide expansion of this species. Moreover, in Europe the distribution range of *P. dominula* also expands rapidly from southern to northern regions. Hence, scientists attempt to find explanations why *P. dominula* is such a good invader (Giovanetti et al. 1996, Judd and Carpenter 1996, Moller 1996, Landolt and Antonelli 1999, Pekkarinen and Gustafsson 1999, Cervo et al. 2000, Pickett and Wenzel 2000, Gamboa et al. 2002, 2004, Liebert et al. 2006, Käfer et al. 2015).

For instance, Bleidorn et al. (2000) speculated that the increasing average annual temperature combined with beneficial periods such as particularly mild winters followed by early warm springs might be one factor of explaining the shift of *P. dominula* to geographic northern areas. But the change of climate worldwide cannot be the only reason for the observation that *P. dominula* is such a good invader of northern territories.

Therefore, some authors favour the idea that nesting in sheltered places within urban environments may be a major benefit for the establishment of *P. dominula* colonies in new regions (Judd and Carpenter 1996, Pickett and Wenzel 2000, Budrys et al. 2013). As a thermophilic species (Blüthgen 1956, Witt 2009), originally native to the warm temperate Mediterranean area (Yamane 1996, Cervo et al. 2000) and because of the incapability to warm their brood (Steiner 1930, reviewed by Jones and Oldroyd 2007) *Polistes* wasps are dependent on warm sheltered nesting sites (Heinrich 1993). The present work with its excessive screening of abandoned (n = 147) and newly built nests (n = 63) of *P. dominula* in the vicinity of Würzburg has in fact disclosed that all nests – without any exception – were found within barns and other constructions related to human habitats. Nests of *P. dominula* usually consist of an unenveloped comb made of chewed plant fibres and a single petiole (**Fig. 43;** Downing and Jeanne 1986, Reeve 1991). Paper wasps impregnate their nests with various oral secretions (West-Eberhard 1969, Wenzel 1996). Nests of *P. dominula*, however, are suspected of having less impregnating oral secretions compared to other *Polistes* species (Pickett and Wenzel 2000). Nesting sites within human constructions would have the advantage to protect against cold temperatures and moisture. Hence, urban habitats with their abundant sheltered sites for nesting together with the availability of plenty food resources might be an essential prerequisite for the successful expansion of *P. dominula* to northern geographic regions.

Fig. 43: Nest construction by *Polistes dominula.*
The nests of *Polistes dominula* wasps are built of chewed wood and a single petiole. The picture at the right side shows a *P. dominula* wasp by collecting building material. The left photo illustrates the single petiole between nesting site and brood comb.

However, numerous additional explanations are discussed in the literature. Some of them describe benefits related to philopatry, polygyny (Cervo et al. 2000), sociality (Moller 1996), aposematism (Cervo et al. 2000, Liebert et al. 2006) and the generalistic diet (Cervo et al. 2000). Other authors describe benefits related to physiology and behaviour like dominance against other sympatric species (Moller 1996, Cervo et al. 2000, Liebert et al. 2006), or benefits in time, such as the reutilization of old nest material (Giovanetti et al. 1996), an earlier start of egg laying (Cervo et al. 2000) and a shorter time for larval development compared to native species

(Cervo et al. 2000, Gamboa et al. 2004, Liebert et al. 2006), as possible reasons for the successful spread. Pickett and Wenzel (2000) provided evidence that *P. dominula* is more productive under laboratory and field conditions than a native species. In their study in the vicinity of Dublin, Ohio (USA), colonies of *P. dominula* were composed of significantly more cells at the end of the season and the workers did emerge significantly earlier compared to the native species *P. metricus.* Furthermore, nests of *P. dominula* showed a higher fecundity in terms of both, total number of individuals produced and the production of gynes. Gamboa et al. (2002, 2004) compared *P. dominula* with the native *P. fuscatus* at the Oakland University preserve (Rochester/Michigan) with respect to the productivity of single- and multiple-foundress colonies, renesting frequencies after natural predation as well as egg, larval and pupal developmental times and found the invasive species to be the winner in all categories.

Even predators and parasites are subject of speculations. Cervo et al. (2000) mentioned that the protected nesting sites might serve as defence against predators and that parasites generally do not exist in new habitats. Liebert et al. (2006) pointed out that in new habitats the threat for *P. dominula* colonies concerning predators could be reduced, because the native predators have only co-evolved with the native wasp species. But Gamboa et al. (2004) contradicted this assumption by presenting evidence that raccoons predate *P. dominula* nests. Additionally, the globalization in terms of the import of goods like fruits from southern Europe seems to be a reason for the rapid worldwide spread of *P. dominula* (Landolt and Antonelli 1999, Pekkarinen and Gustafsson 1999).

In contrast to North America, *P. dominula* was not introduced to Germany recently. Five species of *Polistes* are native (Witt 2009) and *P. dominula* is the only species which exclusively shows a synanthropic behaviour. All other native species prefer completely different habitats and hence are not suitable for a comparison with *P. dominula* **(Table 1).** Therefore, I pursued a different approach to uncover exceptional features of *P. dominula* wasps.

The comprehensive studies about the nesting behaviour of *P. dominula* in Germany have spanned three consecutive summer seasons of the years 2012 - 2014. These screenings have revealed a remarkable flexibility of these wasps in terms of number and sizes of newly founded nests, length of the nesting season and foundress associations at the various developmental stages of establishing new colonies **(Table 5)**. A considerable difference in the number of newly built nests per study year was detected, suggesting that the climatic conditions and the length of the previous winter season might influence the number of surviving queens and, as a consequence, the total number of nests during the following summer months. Furthermore, a notable variation of average nest sizes at the end of the season ranging from 44 cells in 2013 to 125 cells in 2014 **(Table 5; Fig. 14)** and a striking fluctuation in the progress of colony growth was observed. These results underscore the importance of long-term observations of single nesting sites.

During the three summer seasons of the present study, it was noticed that nests consisting of less than 31 cells were usually given up by the queens, or the queens died during the founding phase. Similarly, Pickett and Wenzel (2000) mentioned that the smallest observed field colony of *P. dominula* reached a size of 35 cells per nest. The overall reduced sizes of nests observed in the summer season of 2013 in Würzburg **(Fig. 14)** might have the advantage for the foundresses to consume less energy for the building of nests – as long as the minimal number of cells (30-35) required for survival is maintained.

An average number of about 80 cells from a screening of 147 old abandoned nests **(Fig. 12)** and of 63 newly founded nests **(Table 5)** was estimated at the end of season. This nest size is considerably smaller than that recorded for *P. dominula* colonies in other studies. Thus, a mean cell number of 137 (Ithaca, USA; Tibbetts and Reeve 2003), 149 (Firenze, Italy; Turillazzi 1980), 173 (Dublin, USA; Pickett and Wenzel 2000) and 257 (Michigan, USA; Gamboa et al. 2004) was documented. The original colonizing area of *P. dominula* is the warm temperate Mediterranean region with an approximate length of nesting seasons between 6.5 - 7 months (Yamane 1996).

My observations in the vicinity of Würzburg, Germany (latitude of 49°) indicated an average nesting time of merely 19.7 weeks = 4.6 months **(Table 5; Fig. 14)**, comparable to the assumed short nesting season at the three locations in the United States.

As a primitively eusocial wasp, the cooperation of queens in nest building and brood care of *Polistes* species is common (Reeve 1991). A number of only maximal 4 foundresses per nest was detected during the study period **(Fig. 15)**. This small number of foundresses is in contrast with observations made in other studies. For instance, Zanette and Field (2009) recorded nest buildings of *P. dominula* wasps by 1-10 foundresses in Southern Spain. Furthermore, during this study it became apparent that hibernating queens of *P. dominula* neither preferred multiple nor single founding **(Table 5)**, whereas Turillazzi (1982) determined a ratio of 36% single- versus 64% multiple-foundress colonies. Zanette and Field (2011) described a ratio of 7% single versus 93% multiple founded nests. Presumably, foundress associations are rather affected by nesting place capacity (Reeve 1991) and/or by the total number of successfully hibernating queens at one nesting site. However, a positive effect of multiple founding on the survival of a nest was detected in the present study, **(Table 5; Fig. 15)**, indicating a direct correlation of the number of foundresses and the survival rate. The advantage of multiple-foundress colonies of *P. dominula* for a better survival was also reported by Queller et al. (2000), Tibbetts and Reeve (2003), Leadbeater et al. (2011) and Zanette and Field (2011) and apparently is a general feature of all investigated *Polistes* species (Reeve 1991).

Another benefit of multiple-foundress nests was elucidated by Queller et al. (2000) and Tibbetts and Reeve (2003) who demonstrated that multiple-foundress colonies resulted in an increased productivity as measured by the number of cells per nest. A similar effect was not observed in the present studies. The mean number of cells per nest was not significantly different between single and multiple founding colonies (**Table 5; Fig. 16 A, C, E)**. As a second criterion for productivity, the number of pupae at the end of the founding period was utilized. Sealed brood cells nearly ensure the successful hatching of an adult wasp and affect directly the fitness and productivity of a nest. Yet, again no significant differences between single and multiple founding nests were verified **(Table 5; Fig. 16 B, D, F).**

In summary, the general notion can be supported that *P. dominula* appears to be an extraordinary successful invader of new countries/continents as documented mostly in comparison to another native species (Pickett and Wenzel 2000, Gamboa et al. 2002, 2004). However, the flexible and adaptive behaviour in response to climatic conditions of the preceding winter observed in the present studies is another cause for the success of *P. dominula* in new regions and long-term studies spanning several colony cycles will expand the knowledge about an invasive species.

5.2 THERMOREGULATORY MECHANISMS OF ADULT PAPER WASPS

Thermoregulation in insects can be divided in two broad categories: On the one hand, the thermoregulation of the individual insect and on the other hand – in case of social insects – the thermoregulation of the nest. Thermoregulatory mechanisms on the level of the individual animal play important roles and have been studied extensively in highly social insects (reviewed by Heinrich 1993, reviewed by Jones and Oldroyd 2007). For example, in some social insects like bumble bees or honey bees it is known that these species can heat up their thorax muscles independently from moving their wings. This has two major advantages: Firstly, it is needed for achieving the adequate temperature for flight (a pre-flight warm-up behaviour; Heinrich 1993) especially on cooler days. Secondly, the warm-up behaviour of the thorax without using the wings is a prerequisite for an active warming of the brood (Bujok et al. 2002).

5.2.1 Adult *P. dominula* wasps are able to respond to artificial stressors with elevated thorax temperature

However, there are less data available regarding the thermoregulatory abilities of primitively eusocial insects such as *Polistes* wasps. The experiments presented in this study clearly revealed that individual *P. dominula* wasps recognized smoke and CO_2 and reacted almost instantaneously and simultaneously with an increase of their thorax temperature after the stressor was applied **(Fig. 17; Fig. 18; Fig. 19; Fig. 20 and Table 7).**

Smoke is an important signal for all insects, since it warns them against fire in their habitat. The thorax temperature of the investigated *P. dominula* wasps increased by an average of 1.91°C and the time interval to reach the maximal thoracic temperatures was about 61.5 s **(Table 7).** Whenever smoke was applied for more than 3 s, the animals hectically moved towards the exit and fled, suggesting that *Polistes dominula* wasps exhibited a pre-flight warm-up behaviour in preparation for a quick escape from the simulated fire.

In other social insects, no observations have been published so far about their physical reaction to smoke. Therefore, a similar experiment was carried out with caged honey bees (Höcherl et al. 2013). The analysis by infrared camera images showed a significant increase of the thorax temperatures after the bees were exposed to smoke. Over all 40 series of measurement, a mean temperature enhancement of 3.0°C (± 1.0) was verified from approximately 28°C to 31°C. Honey bees need a thorax temperature of at least 33-35°C for a rapid flight (Esch 1976, Heinrich 1979). The results described above clearly revealed that honey bees are highly stressed by smoke odour. After they have detected smoke, they feed honey and simultaneously heat up their thoraces for being prepared to escape rapidly, like the *Polistes* wasps.

The perception of carbon dioxide (CO_2) by social insects is of particular significance for them. As a result of their respiration, animals always exhale CO_2. Social insects have evolved special antennal receptors for CO_2, but not for oxygen (Lacher 1967, Kleineidam et al. 2000).

Through respiration, O_2 and CO_2 concentrations are inversely coupled to each other. Therefore, insects acquire information about the oxygen content in the nest by using their antennal receptors for CO_2 (Kleineidam et al. 2000). In sheltered nests occupied by honeybees, wasps or bumblebees, where exhaled CO_2 cannot leak easily, perception of CO_2 is important to trigger nest ventilation (Seeley 1974, Weidenmüller et al. 2002). However, for the unenveloped nests utilized by *Polistes* wasps, this explanation does not appear to be appropriate. Therefore, a different interpretation is favoured.

Carbon dioxide is one of the main components of exhaled air of larger animals. As described in the previous chapter, Gamboa et al. (2004) presented evidence that raccoons predate *P. dominula* nests in the USA. In addition, different bird species are natural enemies of this paper wasp species (N. Höcherl, personal observation). Therefore, it could be an advantage for these wasps to be able to perceive CO_2 in order to detect predators in due time to initiate defence reactions. Initially, the Polistes wasps reacted to the application of CO_2 nearly in the same way as to the stressor "smoke" **(Fig. 17; Fig. 18; Fig. 19; Fig. 20; Table 7).** The analysis of 20 data sets revealed that the average rise of the thorax temperature was about 1.6°C and that the time to reach the highest thorax temperature was 65.0 s **(Table 7).** Yet, whenever CO_2 was applied for a period longer than 3 s, or if CO_2 was administered in a breath-like way, the wasps exhibited a hectical movement and started flying and circling around in the direct environment of the nest but did not flee. These observations raised the question by which means the wasps responded to CO_2.

In social wasps, alarm substances were first detected by Maschwitz (1964). Since then, the existence of alarm pheromones was proven for many highly social wasp species (Sledge et al. 1999). The observed reactions were mostly related to venom secretions. Similarly, for some primitively social wasp species *(P. canadensis , P. exclamans, P. fuscatus)* it has been demonstrated that an alarm pheromone exists (Jeanne 1982, Post et al. 1984). In many other species, the existence of alarm pheromones has been proposed, but evidence is still lacking

(Sledge et al. 1999). Based on the results of the present work, it could be assumed that an alarm substance is also present in *P. dominula* wasps. The detection of the sudden increase of CO_2 in the direct proximity of the nest possibly alerts the wasps resulting in the release of an alarm pheromone. As a consequence, the wasps start to heat up their thoraces in preparation for defending the nest.

Heinrich (1979, 1984) suggested that a higher thoracic temperature could be seen in connection with nest defence. He found that honey bees of the species *Apis mellifera mellifera* (former known as the European honey bee) and *Apis mellifera adansonii* (the African honey bee) reached highest thorax temperatures (36 - 38°C) while leaving the hive and during an attack (Heinrich 1979). Based on his results, he concluded that this can be seen in connection with a pre-flight warm-up behaviour. Additionally, Heinrich (1984) found that attacking *Dolichovespula maculata* hornets consistently expressed the highest thorax temperature, too. He concluded that this could be very functional during a nest defence situation in terms of a fast and manoeuvrable flight. Weiner et al. (2010) speculated that maintaining elevated body temperatures prior to aggressive interactions with nest intruders might be more costly than had been previously thought (Weiner et al. 2009). The observations in the present work clearly imply that *Polistes* wasps react with a pre-flight warm-up behaviour upon exposition to smoke or CO_2 in order to quickly escape or to defend their nest against aggressors.

5.2.2 The dominant queen reacts with increased thorax temperature to attacks of subordinate queens

As mentioned in the preceding chapter, in members of primitively eusocial wasps foundress associations are quite normal (West 1967, Reeve 1991, this work). If a colony is founded by more than one queen, a linear dominance hierarchy emerges. *Polistes dominula* was the first species in which dominance hierarchies were described (summarized by Pardi 1996). One foundress becomes dominant and lays most of the eggs, while the subordinate queen(s) take care of the riskier work like foraging (Queller et al. 2000, reviewed by Jandt et al. 2014). For developing a linear dominance hierarchy, the involved queens exhibit different stages of aggressive behaviour (West-Eberhard 1969, reviewed by Jandt et al. 2014). After a hostile posture towards each other, they may bite legs or antennae. If the conflict escalates, there may be a so-called “falling flight” in which both queens fall from the nest (**Fig. 44**).

At the six study sites, the number of foundresses per nest ranged between one and four. An observation of a total of 29 multiple-foundress nests was possible during the three study years. Five of these nests were built at locations that made recordings with an infrared camera possible. The analysis of 12 dominance battles where the conflict did not escalate and the queens remained on the nest all the time revealed that the dominant queens increased their thorax temperature on average by 2.23°C (max. 5.20°C; min. 1.10°C; **Fig. 22**).

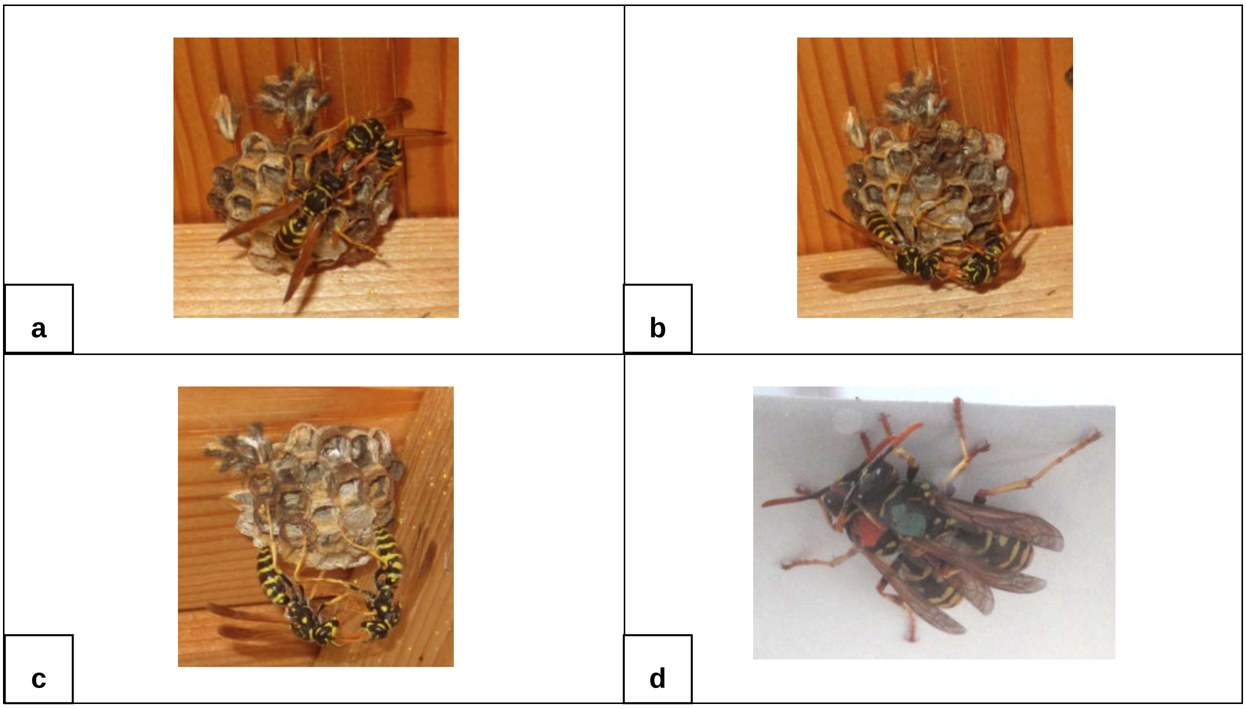


Fig. 44: A dominance battle of *P. dominula* queens in a sequence of photographic illustrations.

(a) The fight between two queens begins with a hostile posture towards each other. (b) The aggression among them increases by trying to bite each other’s legs or antennae. (c) The fight approaches its climax and may end with the “falling flight”, if both queens lose their grip. (d) At the end of the fight, the loser bows herself to the victor of the fight by occupying a specific posture – in this special case the red marked queen bows herself to the green marked queen by permitting her to stay atop of herself.

In contrast, the subordinate queens showed a slight increase of their thorax temperature in only three out of twelve battles. Additionally, it was not observed that the dominant queens moved their wings or legs more than the subordinate queens during the fights on the nest. One explanation could be that the warm-up behaviour of the dominant queen is to be seen in connection to nest defence (as discussed in the response of *Polistes* wasps to CO_2). The dominant queen apparently defends not only herself but also her nest. This assumption would be in accordance with the hypotheses of Heinrich (1979, 1984) and Weiner et al. (2010) that attacking social insects need higher thoracic temperatures for being much faster and more manoeuvrable during flight and that these wasps show this behaviour prior to aggressive interactions with conspecifics, too.

In summary, the present work demonstrates for the first time that thermoregulation of individual adult *P. dominula* wasps on the nest has more important functions than previously known.

5.3 THERMOREGULATORY MECHANISMS OF *POLISTES DOMINULA* NESTS ARE MORE DIVERSE THAN DESCRIBED BEFORE

The nests of social insects serve as incubators for raising brood as well as protection against enemies and temperature extremes (Heinrich 1993). Temperatures below 30°C are generally considered to inhibit the growth and may cause developmental damage in bumble bee species (Heinrich 2001). In honey bee brood, permanent temperatures below the optimal 34-35°C can lead to massive damages which are not only reflected in negative physical but also in negative behavioural effects by the adults (Tautz 2003, Groh et al. 2004, Jones et al. 2005). Therefore, in most social insects the brood rearing temperature is one of the most controlled parameters (Medrzycki et al. 2010).

Many data are available regarding the thermoregulation of nests of social insects, including copious reviews (see Heinrich 1993). Although repeatedly emphasized that our information about the thermoregulation in social wasps is based on studies of the paper wasp *Polistes gallica* (former name of *P. dominula*; Heinrich 1993, Jones and Oldroyd 2007), a closer look revealed that this knowledge is based on three main reports that were published more than eighty years ago (Steiner 1930, 1932, Weyrauch 1936). Since nowadays novel techniques are available, such as data logger with a minimum of measurement errors and the infrared camera, a re-investigation of the thermoregulatory mechanisms of *P. dominula* nests appeared to be necessary.

5.3.1 Adult *P. dominula* wasps regulate the temperature of their nest by effective cooling

Known active thermoregulatory systems to raise the nest temperatures are generating metabolic heat as well as the direct incubation of the brood cell (Jones and Oldroyd 2007). Clustering or the possibility to generate metabolic heat is very common in social insects and one of the simplest methods to raise the temperature in the nest (Ishay 1973, reviewed by Heinrich 1993, Bujok et al. 2002, Yamane et al. 2009). Particularly honey bee species are able to generate metabolic heat by contracting and releasing their flight muscles while the latter are decoupled from the wings (Fahrenholz et al. 1989). Even some wasp species are capable of generating metabolic heat while clustering on the surface of brood cells (Ishay 1973, Yamane et al. 2009). Larvae of the hornet *Vespa orientalis* are also thought to play a role in warming by activating their muscles (Ishay and Barenholz-Paniry 1995).

However, Jeanne and Morgan (1992) assumed that the small colonies of *Polistes* wasps do not generate much metabolic heat. Steiner (1930) showed that the metabolic heat of the brood of *P. dominula* exerts no influence on the nest temperature. The experiments in the present work support this observation. The non-active nests (without brood and adults) remained as warm or cool as the active nests (with brood and adults) on cool days (15°C and 20°C). Therefore, another statement of Steiner (1930) can also be accepted: The metabolic heat of the adults does not influence the nest temperature **(Figs. 26 and 27)**.

Similar to Steiner´s work (1930, 1932), no active incubation of the brood by the adult *Polistes dominula* wasps could be detected in the present study. Warming of the brood is known from many different social insect species (reviewed by Heinrich 1993, reviewed by Jones and Oldroyd 2007). In hornets, workers incubate pupae by entering empty cells directly beside the respective cell (Ishay 1973), especially if late-stage pupae elicit the need for enhanced temperatures by releasing a pheromone (Jeanne 1996). During this work, I myself was able to observe this behaviour in *Vespa crabro* – the native European hornet **(Fig. 45)**. Entering empty cells for warming neighbouring cells was also shown for honey bees (Kleinhenz et al. 2003).

Another incubation behaviour has been reported for honey bee workers. These workers press their warm thoraces onto the caps of sealed brood cells in order to maintain a constant high brood temperature (Bujok et al. 2002). Bumble bee queens and queens of some wasp species are known to incubate their first brood cells in spring by insulating, active warming and curling over the cells (Makino and Yamane 1980, Heinrich 1993). After some workers hatched, they took care of the brood including the warming almost like the described behaviour for honey bees.

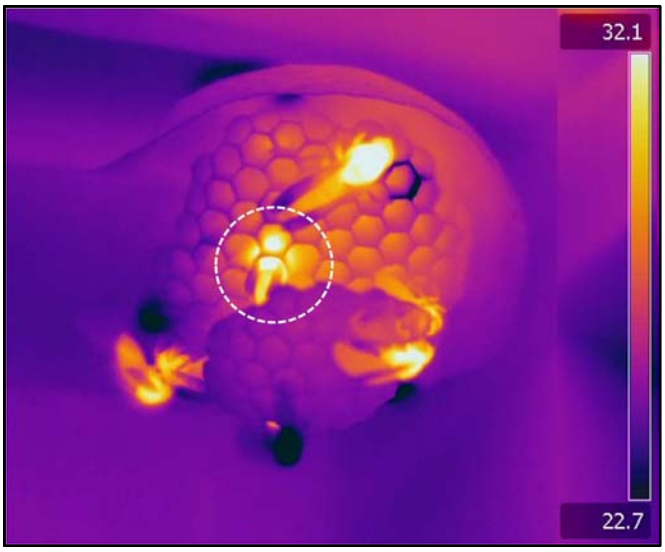


Fig. 45: Thermographic image of a hornet nest ***(Vespa crabro).***
The nest was a subsidiary nest which was built in July 2012 at the study site no. 1. The white dotted circle marks a hornet worker that incubates the surrounding cells by entering an empty cell. The temperature scale for this picture is shown at the right side.

Active thermoregulatory mechanisms to lower the nest temperature are wing fanning and water evaporation (Jones and Oldroyd 2007). The studies of the present work clearly revealed that *P. dominula* wasps are extraordinarily efficient in cooling the nest **(see 4.3.1 and 4.3.2)**. Steiner (1930) proved in his work that the fanning behaviour starts when the nest temperature ranges between 31.50°C and 35.25°C. In the present study that involved the observation of 63 newly founded nests, fanning behaviour directly on the nest was almost undetectable. Especially wing fanning for cooling, only four cases were observed **(see 4.3.2.2).** The hottest temperature where wing fanning was detected was 37.94°C – which is about 2.7°C above Steiner´s (1930) measurements.

However, Steiner (1930) himself mentioned that the observation of this behaviour is not easy, because wing fanning is also used during excitation. The differentiation between fanning for cooling and fanning because of excitation is virtually impossible at higher nest temperatures. In addition, he also found out that the cooling by ventilation only slightly lowers the nest temperature. This cannot be confirmed in the present study because no effect of ventilation on the nest temperature was detected **(see 4.3.2.2).** Thus, Steiner´s measurements and the results of this work could also mean that the fanning behaviour in terms of cooling a nest was overrated in some other social insects so far. It could be possible that fanning is more significant for the regulation of other important factors like the concentration of CO_2 (as discussed in 5.2.1) especially in enveloped nests as proven for some stingless bees (Moritz and Crewe 1988)

Steiner (1930, 1932) concluded from his experiments that fanning behaviour only attained its real significance in conjunction with cooling by water evaporation. In the present work, water evaporation is the most effective feature for cooling **(Figs. 33 and 34)**. An average decrease of temperature of a single cell of about 8°C and a mean duration of 7 min until the cell reached its basic temperature, very clearly demonstrated the role of water in nest cooling. Furthermore, the start of cooling by water evaporation was detected above maximum outside temperatures of 25°C or nest temperatures above 35°C **(Figs. 25 a, 29, 30 and 31)**. Steiner (1930) detected the start of water transport at nest temperatures between 34°C and 37.5°C, which is in accordance to this study. Likewise, his result that the nest temperature is stabilized within the same temperature range could be partially confirmed **(Figs. 28, 29 and 30).**

A stable species-specific brood nest temperature is essential for an optimal development of the immature (Jones and Oldroyd 2007). The use of water for cooling is also known for all honey bee species as well as for some hornet species (Lindauer 1954, Dyer and Seeley 1991, Ishay and Barenholz-Paniry 1995, Jones and Oldroyd 2007), but not for bumble bees as overheating is probably not a common problem – due to their chosen subterranean nesting sites (Heinrich 1993, Jones and Oldroyd 2007).

5.3.2 Passive mechanisms of regulating the nest temperature of *P. dominula* were underestimated so far

The known passive thermoregulatory mechanisms to influence the nest temperature are nest site selection, nest orientation and nest architecture (Jones and Oldroyd 2007). The first step in nest building is site selection (Wenzel 1996). Finding suitable nesting sites is most important especially for species that have only a limited capacity for regulating the nest temperature (Heinrich 1996). Through the choice of proper nesting sites, substantial energy saving is possible (Heinrich 2001). Social wasps can be found in very different locations, in tree hollows as well as underground and both in enclosed and open nests (Ishay 1973). Similar to bumble bee queens (Heinrich 1996), queens of *P. dominula* spend a lot of time and energy searching for a suitable place for their nests and often prefer sites used the previous year (N. Höcherl, pers. observation). Jeanne and Morgan (1992) hypothesize that the search for microclimatically proper nesting sites has led to the evolution of philopatry.

As revealed in the present work, the choice of the nesting site is very important for *P. dominula* queens **(see 4.3.4)**. Different nesting sites can imply great variations in temperature during similar environmental conditions **(Figs. 39 and 40)**. Furthermore, within one suitable nesting site the microclimate can vary **(Fig. 41)**. These variations in temperature can lead to massive energetic costs (for cooling activities), if wasps have chosen nesting sites which heat up extraordinarily. Jeanne and Morgan (1992) assumed that *Polistes* colonies are subjected to fluctuations in ambient temperatures and that in temperate regions the ambient temperatures are below the optimum for brood development and survival. Steiner (1930) concluded by pure observation that the only heat sources for a *Polistes dominula* nest is the warmth of the direct ambience, especially the ambient air and the insolation. For *P. fuscatus* it is known that temperature influences the nest site choice and the reproductive strategy (Jeanne and Morgan 1992). Therefore, it is quite possible that the ambient temperature plays a crucial role in nest site choice, even in *P. dominula*.

Nest orientation as possible thermoregulatory mechanism was described in different termite and ant species. It is known that some termites adjust their nests in a certain cardinal direction and thus affect the nest temperatures (Jones and Oldroyd 2007). The Australian "compass termites" *(Amitermes meridionalis* and *Amitermes laurensis)* build wedge-shaped mounds. The long axis of these mounds is oriented north-south (Grigg and Underwood 1977, Jacklyn 1992). These termites influence the amount of insolation absorbed by the mound. During the morning, the nests have the advantage of a rapid warm-up, but during the hot time of the day – until the sun sets – the temperature reached a plateau. Fire ants *(Solenopsis invicta)* use the same mechanism as the compass termites. The mounds of these ants are oval shaped and the long axis is oriented north-south. The greatest area of the mound surface is facing the sun in the morning and late afternoon, but never when the sun is at its highest point (Jones and Oldroyd 2007).

In wasp species, this kind of passive thermoregulation has not yet been documented **(Table 3)**, even if there were some signs and notes in this regard. Steiner (1932) found nests of *P. dominula* only on the eastern and southern sides of barns and other buildings. This was also observed in the present work (N. Höcherl, pers. observation). The wasps seem to avoid north and west-facing surfaces. The advantage could be to use – similar to the fire ants and compass termites – the rough warm-up of the nest during the morning. However, in contrast to the last-mentioned species, *P. dominula* wasps seem to need the insolation as much as possible by preferring south-oriented nesting sites.

Animals are sometimes true artists in building their nests (Arndt 2013) and especially social insects are well known for their sophisticated nests (Heinrich 1996). Honey bees actually produce their building material (wax) themselves. Therefore, they belong even in this respect to the elite among the social insects (Tautz 2007). Moreover, the nesting in chosen cavities causes the insulation of nests in bees (Jones and Oldroyd 2007).

In addition, bumble bees build a "roof" made of wax over the nest to preserve metabolic heat (Heinrich 1996). Similarly, complex layers surrounding the nest are also known in many stingless bee species (reviewed by Jones and Oldroyd 2007).

In wasps, nest architecture is very variable **(Fig. 5)**. Species of Vespinae often construct their nest in cavities and/or they are enclosed by a paper layer (called envelope). Klingner et al. (2006) were able to prove that the construction of nests of the European hornet *(Vespa crabro)* is insulating but does not help buffering variations of ambient temperature. The authors conclude that this explains why hornets prefer nesting in cavities for additional thermoregulation. A very interesting feature in nest thermoregulation by nest architecture was found in the oriental hornet *(Vespa orientalis)*. The silk caps of cells containing pupae store "electrical charge" during warm phases and release that energy at cooler parts of the day (Ishay and Barenholz-Paniry 1995). Some Polistinae wasps, especially of the genus *Polybia* from South America built nests with a layer surround the brood comb. Hozumi et al. (2010) were able to show that the temperature in an abandoned nest of *Polybia scutellaris* follows mainly the changes in ambient temperature. This result indicates that the envelope has no or only small influence on nest temperature. However, in another *Polybia* species *(P. occidentalis)* empty combs were detected. These unused combs were shown to help keeping a higher nest temperature and to protect the brood from extreme changes of the ambient temperature (Hozumi et al. 2008).

Polistes wasps neither nest in cavities nor build enveloped nests. Furthermore, the nests of this species consist of only one single comb. Therefore, other mechanisms must have evolved. During the last 40 years, the nest architecture of some *Polistes* species was examined. In all investigated nests of different species, it was found that they built short empty cells in the periphery and extraordinarily long cells in the centre of the nest (*P. chinensis:* Yamane 1972; *P. biglumis:* Lorenzi and Turillazzi 1986; *P. riparius:* Yamane et al. 1998, Hozumi and Yamane 2001). Lorenzi and Turillazzi (1986) regard this aspect as prevention from being damaged by rain and hailstones, whereas Yamane et al. (1998) interpreted it to be an adjustment to colder climate. In the 70´s, this special way of constructing a nest was recognized by Yamane and Kawamichi (1975). They suggested that this structure may serve as an air chamber similar to the envelope of nests of Vespine wasps. Therefore, they called it a "functional envelope".

In the present study, a classical functional envelope – as defined by Yamane and Kawamichi (1975) – could not be detected. In some nests, the outer cells were longer than those in the centre of the nest **(Fig. 46 a)**, which contradicts the typical structure of a functional envelope. Furthermore, the peripheral cells were mostly occupied by brood **(Fig. 46 b)**. Nevertheless, the edges of *P. dominula* nests were found to be warmer than the inner cells during the day and cooler in the evening **(Fig. 23)**. Apparently, another type of functional envelope is utilized in the architecture of *P. dominula* nests.

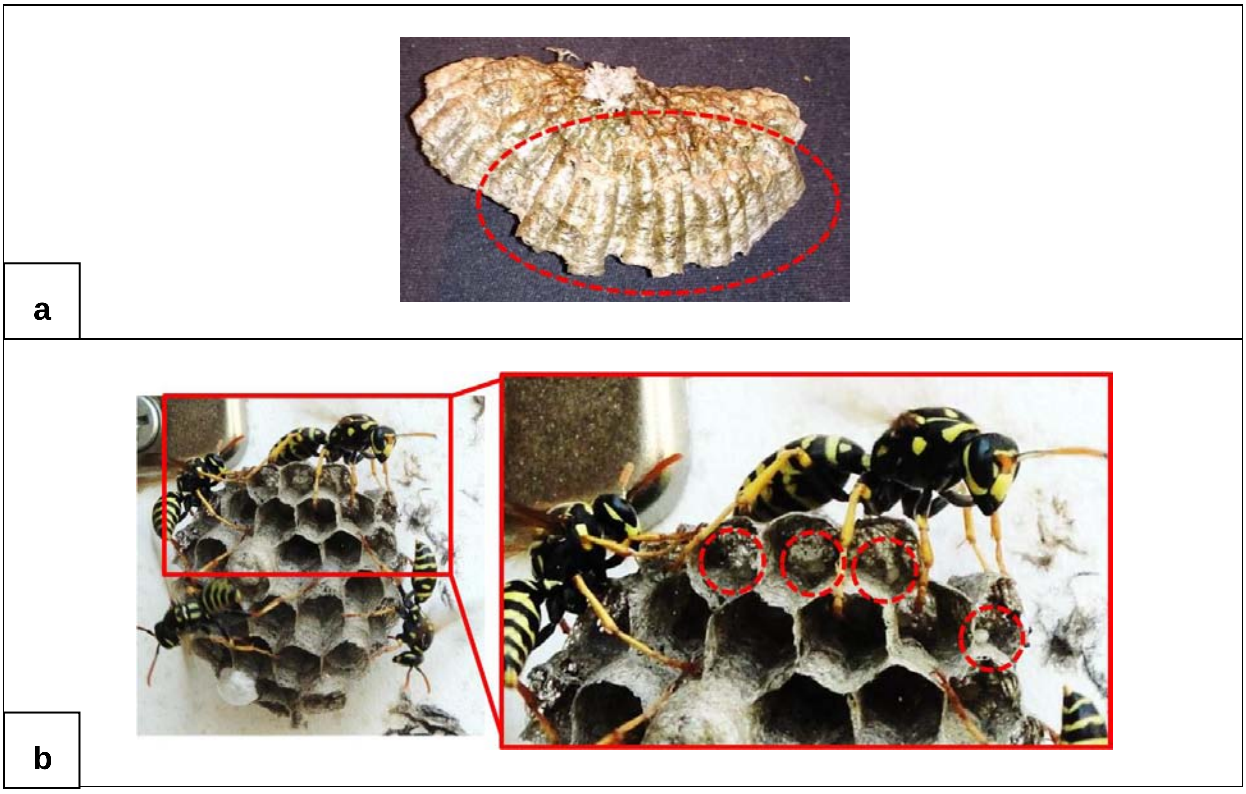


Fig. 46: Nest architecture of *P. dominula* wasps.
The pictures indicate the absence of a classical "functional envelope" in *P. dominula* nests. (a) Photo of an old abandoned nest which shows that the cells in the periphery are partly longer than in the centre of the nest. (b) On the left side, the whole nest is shown; on the right side, an image section of the nest is enlarged to exemplify that peripheral cells are containing eggs.

Steiner (1930) mentioned that the heat capacity of the nest cannot virtually exercises an impact on the social heat balance. Furthermore, it was suggested that no retention of warmth can take place, because of the absence of envelopes (Steiner 1930, Jeanne and Morgan 1992). In the present study, it was demonstrated that non-active nests follow the ambient temperature **(Figs. 24 b, 25 b, 26, 27, 28, 29 and 30)**. Hence, the assumption that nests of *Polistes* wasps are subjected to variations in ambient temperatures is basically correct (Steiner 1930, Jeanne and Morgan 1992).

However, non-active nests show partly great differences to the roof temperatures **(i. e. Figs. 25 b and 28**, on average 6.24°C and 5.75°C, respectively). This indicates that some passive thermoregulatory mechanisms must occur in the nests of this species. A nest of *Polistes* is mostly described as nests that usually consist of a single petiolate (stelocyttarus) and an unenveloped comb (Reeve 1991). Stelocyttarus nests are connected to the base (in *P. dominula* often roofs) by the stalk-like structure called pedicel (Camazine et al. 2001). Taking a closer look to the pedicels which are constructed by the queens in the early stages of the nests **(Fig. 47)**, it seems that they always have about the same length.

Fig. 47: Founding of the nest by a *P. dominula* queen.
The images show a queen building the pedicel (left) and the construction of the first two cells of the nest (right).

A measurement of 20 pedicels revealed a mean length of 0.34 cm (± 0.08) with a maximum of 0.5 cm and a minimum of 0.2 cm. The pedicels could have an important influence on the nest temperature, because between nests and wall an air-filled gap emerges. This gap could achieve a physical insulation of the nest.

In addition, the combs constructed by *Polistes dominula* are always built in a horizontal orientation which means that the cells are directed downwards. Through the laws of physics, warm air always rises to the top. During the cooler evening and night hours, the warm air accumulates in the cells and thus warms the nest **(Figs. 24, 25, 35, 36 and 37)**.

In summary, it is indisputable that *P. dominula* wasps prefer microclimatically perfect sites for their nests to develop optimal and that their abilities to regulate the nest temperature are limited. However, from the thermoregulatory point of view *P. dominula* nests are not as unprotected as assumed so far.

REFERENCES

Arndt I. (2013). Architektier. Knesebeck GmbH & Co. Verlag KG, München.

Bleidorn C., Lauterbach K. E., Schulze W., Venne C. (2000) Über die weitere Ausbreitung der Französischen Feldwespe *Polistes dominulus* (Christ, 1791) in Ostwestfalen (Hymenoptera, Vespidae). Mitteilungsblatt der Arbeitsgemeinschaft ostwestfälisch-lippischer Entomologen **16/2:** 35-39.

Blüthgen P. (1956) Über einige *Polistes*-Arten der Zool. Staatssammlung in München. Nachrichtenblatt bayerischer Entomologen **5:** 81-86.

Borkent C. J., Cannings R. A. (2004) *Polistes dominulus* (Christ) (Hymenoptera: Vespidae: Polistinae) in British Columbia: first collection records of an invasive European paper wasp in Canada. Journal of the Entomological Society of British Columbia **101:** 149-150.

Budrys E., Budriene A., Gliwa B. (2013) Distribution of paper wasps (*Polistes* Latreille, 1802) in Lithuania (Hymenoptera: Vespidae). Naujos Ir Retos Lietuvos Vabdziu Rusys **25:** 79-82.

Bujok B., Kleinhenz M., Fuchs S., Tautz J. (2002) Hot spots in the bee hive. Naturwissenschaften **89:** 299-301.

Camazine S., Deneubourg J.-L., Franks N. R., Sneyd J., Theraulaz G., Bonabeau E. (2001) Self-Organisation in Biological Systems. New Jersey, Woodstock. Princeton University Press.

Carpenter J. M. (1996) Distributional Checklist of Species of the Genus *Polistes* (Hymenoptera: Vespidae; Polistinae, Polistini). American Museum Novitates **3188:** 1-40.

Cervo R., Zacchi F., Turillazzi S. (2000) *Polistes dominulus* (Hymenoptera, Vespidae) invading North America: some hypotheses for its rapid spread. Insectes Sociaux **47:** 155-157.

Coelho J. R., Ross A. J. (1996) Body temperature and thermoregulation in two species of yellowjackets, *Vespula germanica* and *V. maculifrons*. Journal of Comparative Physiology B **166:** 68-76.

Coelho J. R. (2001) Behavioral and physiological thermoregulation in male cicada killers *(Sphecius speciosus)* during territorial behavior. Journal of Thermal Biology **26:** 109-116.

Downing H. A., Jeanne R. L. (1986) Intra- and interspecific variation in nest architecture in the paper wasp *Polistes* (Hymenoptera, Vespidae). Insectes Sociaux **33:** 422-443.

DWD: Deutscher Wetterdienst. www.dwd.de

Dyer F. C., Seeley T. D. (1991) Nesting behaviour and the evolution of worker tempo in four honey bee species. Ecology **72:** 156-170.

Eardley C., Koch F., Wood A. R. (2009) *Polistes dominulus* (Christ, 1791) (Hymenoptera: Polistinae: Vespidae) newly recorded from South Africa. African Entomology **17(2):** 226-227.

Esch H. (1976) Body temperature and flight performance of honey bees in a servomechanically controlled wind tunnel. Journal of Comparative Physiology **109:** 264-277.

Fahrenholz L., Lamprecht I., Schricker B. (1989) Thermal investigation of a honey bee colony: thermoregulation of the hive during summer and winter and heat production of members of different bee castes. Journal of Comparative Physiology B **159:** 551-560.

Field J., Solis C. R., Queller D. C., Strassmann J. E. (1998) Social and genetic structure of paper wasp cofoundress associations: Tests of reproductive skew models. The American Naturalist **151:** 545-563.

Gamboa G. J., Greig E. I., Thom M. C. (2002) The comparative biology of two sympatric paper wasps, the native *Polistes fuscatus* and the invasive *Polistes dominulus* (Hymenoptera, Vespidae). Insectes Sociaux **49:** 45-49.

Gamboa G. J., Noble M. A., Thom M. C., Togal J. L., Srinivasan R., Murphy B. D. (2004) The comparative biology of two sympatric paper wasps in Michigan, the native *Polistes fuscatus* and the invasive *Polistes dominulus* (Hymenoptera, Vespidae). Insectes Sociaux **51:** 153-157.

Giovanetti M. R., Cervo R., Turillazzi S. (1996) Comb reutilization in *Polistes dominulus* (Hymenoptera: Vespidae). Insect Social Life **1:** 101-105.

Grigg G. C., Underwood A. J. (1977) An analysis of the orientation of "magnetic" termite mounds. Australian Journal of Zoology **25:** 87-94.

Groh C., Tautz J., Rössler W. (2004) Synaptic organization in the adult honey bee brain is influenced by brood-temperature control during pupal development. Proceedings of the National Academy of Sciences **101:** 4268-4273.

Hathaway M. A. (1981) *Polistes gallicus* in Massachusetts (Hymenoptera: Vespidae). Psyche **88:** 169-173.

Hathaway M. A. (1982) *Polistes gallicus* in North America. Specos **5:** 9-10.

Heinrich B. (1979) Thermoregulation of African and European honeybees during foraging, attack, and hive exits and returns. Journal of Experimental Biology **80:** 217-229.

Heinrich B. (1984) Strategies of thermoregulation and foraging in two vespid wasps *Dolichovespula maculata* and *Vespula vulgaris*. Journal of Comparative Physiology B **154:** 175-180.

Heinrich B. (1993) The Hot Blooded Insects: Strategies and Mechanisms of Thermoregulation. Harvard University press, Cambridge.

Heinrich B. (1996) The Thermal Warriors: Strategies of Insect Survival. Harvard University press, Cambridge Massachusetts, London.

Heinrich B. (2001) Der Hummelstaat – Überlebensstrategien einer uralten Tierart. Econ Ullstein List Verlag GmbH & Co. KG, München.

Hesler L. S. (2010) *Polistes dominula* (Christ, 1791) (Hymenoptera: Vespidae: Polistinae) found in South Dakota, USA. Insecta Mundi **0145:** 1-3.

Himmer A. (1927) Ein Beitrag zur Kenntnis des Wärmehaushaltes im Nestbau sozialer Hautflügler. Zeitschrift für vergleichende Physiologie **5**: 375-389.

Himmer A. (1936) Das Verhalten sozialer Wespen bei Nestüberhitzung. Zeitschrift für vergleichende Physiologie **23 (1):** 51-63.

Höcherl N., Kennedy S., Tautz J. (2013) Smoke causes "Hot Bees". Poster at the conference SCIX, Würzburg, Germany

Hozumi S., Yamane S. (2001) Incubation ability of the functional envelope in paper wasp nests (Hymenoptera, Vespidae, *Polistes*): I. Field measurements of nest temperature using paper models. Journal of Ethology **19:** 39-46.

Hozumi S., Kudô K., Zucchi R. (2008) Promotion of thermoregulatory insulation in nests of neotropical wasps by building extra-combs with empty cells. Neotropical Entomology **37 (2):** 159-166.

Hozumi S., Mateus S., Kudô K., Kuwahara T., Yamane S., Zucchi R. (2010) Nest thermoregulation in *Polybia scutellaris* (White) (Hymenoptera: Vespidae). Neotropical Entomology **39 (5):** 826-828.

Hunt J. H. (2006) Evolution of castes in *Polistes*. Annales Zoologici Fennici **43:** 407-422.

Ishay J. S. (1973) Thermoregulation by social wasps: behavior and pheromones. Transactions of the New York Academy of Sciences Journal **35:** 223-236.

Ishay J., Barenholz-Paniry V. (1995) Thermoelectric Effect in Hornet *(Vespa orientalis)* Silk and Thermoregulation in a Hornet´s Nest. Journal of Insect Physiology **41(9):** 753-759.

Jacklyn P. M. (1992) "Magnetic" termite mound surface are oriented to suit wind and shade conditions. Oecologia **91:** 385-395.

Jacobson R. S. (1986) *Polistes dominulus* in New Jersey. Sphecos **13:** 22.

Jacobson R. S. (1991a) *Polistes dominulus* spreading in USA. Sphecos **21:** 14.

Jacobson R. S. (1991b) *Polistes dominulus* spreads. Sphecos **22:** 9.

Jandt J. M., Tibbetts E. A., Toth A. L. (2014) *Polistes* paper wasps: a model genus for the study of social dominance hierarchies. Insectes Sociaux **61:** 11-27.

Jeanne R. L. (1982) Evidence for an alarm substance in *Polistes canadensis*. Experientia **38:** 329-330.

Jeanne R. L., Morgan R. C. (1992) The influence of temperature on nest site choice and reproductive strategy in a temperate zone *Polistes* wasp. Ecological Entomology **17:** 135-141.

Jeanne R. L. (1996) The evolution of exocrine gland function in wasps. Pages 144-160 *in* S. Turillazzi, and M. J. West-Eberhard, editors. Natural History and Evolution of Paper-Wasps. Oxford University Press, Oxford U. K.

Jones J. C., Helliwell P., Beekman M., Maleszka R., Oldroyd B. P. (2005) The effects of rearing temperature on developmental stability and learning and memory in the honey bee, *Apis mellifera*. Journal of Comparative Physiology A **191:** 1121–1129.

Jones J. C., Oldroyd B. P. (2007) Nest Thermoregulation in Social Insects. Advances in Insect Physiology **33:** 153-191.

Judd T. M., Carpenter J. M. (1996) *Polistes dominulus* (Hymenoptera: Vespidae) Found in Michigan. The Great Lakes Entomologist **29(1):** 45-46.

Käfer H., Kovac H., Oswald B., Stabentheiner A. (2015) Respiration and metabolism of the resting European paper wasp *(Polistes dominulus)*. Journal of Comparative Physiology B doi: 10.1007/s00360-015-0915-7.

Kleineidam C., Romani R., Tautz J., Isidoro N. (2000) Ultrastructure and physiology of the CO_2 sensitive sensillum ampullaceum in the leaf-cutting ant *Atta sexdens*. Arthropod Structure & Development **29:** 43-55.

Kleinhenz M., Bujok B., Fuchs S., Tautz J. (2003) Hot bees in empty broodnest cells: heating from within. The Journal of Experimental Biology **206:** 4217-4231.

Klingner R., Richter K., Schmolz E., Keller E. (2005) The role of moisture in the nest thermoregulation of social wasps. Naturwissenschaften **92:** 427-430.

Klingner R., Richter K., Schmolz E. (2006) Strategies of social wasps for thermal homeostasis in light paper nests. Journal of Thermal Biology **31:** 599–604.

Kovac H., Schmaranzer S. (1996) Thermoregulation of honeybees *(Apis mellifera)* foraging in spring and summer at different plants. Journal of Insect Physiology **42:** 1071-1076.

Kovac H., Stabentheiner A. (1999) Effect of food quality on the body temperature of wasps *(Paravespula vulgaris)*. Journal of Insect Physiology **45:** 183-190.

Kovac H., Stabentheiner A., Schmaranzer S. (2009) Thermoregulation of water foraging wasps (*Vespula vulgaris* and *Polistes dominulus*). Journal of Insect Physiology **55:** 959-966.

Lacher V. (1967) Verhaltensreaktionen der Bienenarbeiterin bei Dressur auf Kohlendioxid. Zeitschrift für vergleichende Physiologie **54:** 75-84.

Landolt P. J., Antonelli A. (1999) The paper wasp *Polistes dominulus* (Christ) (Hymenoptera: Vespidae) in the state of Washington. Pan-Pacific Entomologist **75:** 58–59.

Lauterbach K. E. (1996) Eine Feldwespe in Ostwestfalen (hym., Vespidae). Mitteilungsblatt der Arbeitsgemeinschaft ostwestfälisch-lippischer Entomologen **12:** 19-21.

Leadbeater E., Carruthers J. M., Green J. P., Rosser N. S., Field J. (2011) Nest Inheritance Is the Missing Source of Direct Fitness in a Primitively Eusocial Insect. Science **333:** 874-876.

Liebert A. E., Gamboa G. K., Stamp N. E., Curtis T. R., Monnet K. M., Turillazzi S., Starks P. T. (2006) Genetics, behaviour and ecology of a paper wasp invasion: *Polistes dominulus* in North America. Annales Zoologici Fennici **43:** 595-624.

Lindauer M. (1954) Temperaturregulierung und Wasserhaushalt im Bienenstaat. Zeitschrift für vergleichende Physiologie A **36:** 391-432.

Lorenzi M. C., Turillazzi S. (1986) Behavioural and ecological adaptations to the high mountain environment of *Polistes biglumis bimaculatus*. Ecological Entomology **11:** 199-204.

Makino S., Yamane S. (1980) Heat production by the foundress of *Vespa simillima,* with description of its embryo nest (Hymenoptera: Vespidae). Insecta Matsumurana. Series Entomology. New series. **19:** 89 -101.

Maschwitz U. (1964) Alarm substances and alarm behavior in social Hymenoptera. Nature **204:** 324-327.

Medrzycki P., Sgolastra F., Bortolotti L., Bogo G., Tosi S., Padovani E., Porrini C., Sabatini A. G. (2010) Influence of brood rearing temperature on honey bee development and susceptibility to poisoning by pesticides. Journal of Apicultural Research **49(1):** 52-59.

Menke A. S. (1993) *Polistes dominulus* in Conneticut. Specos **24:** 12.

Moller H. (1996) Lessons for invasion theory from social insects. Biological Conservation **78:** 125-142.

Moritz R. F. A., Crewe R. M. (1988) Air ventilation in nests of two African stingless bees *Trigona denoiti* and *Trigona gribodoi*. Experientia **44:** 1024-1027.

Nonacs P., Liebert A. E., Starks P. T. (2006) Transactional Skew and Assured Fitness Return Models Fail to Predict Patterns of Cooperation in wasps. The American Naturalist **167:** 467-480.

Pardi L. (1996) *Polistes:* analysis of a society. Pages 1-17 *in* S. Turillazzi and M. J. West-Eberhard, editors. Natural History and Evolution of Paper-Wasps, Oxford University Press, Oxford.

Pekkarinen A., Gustafsson B. (1999) The *Polistes* species in northern Europe (Hymenoptera: Vespidae). Entomologica Fennica **10:** 191-197.

Pickett K. M., Wenzel J. W. (2000) High Productivity in haplometrotic colonies of the introduced paper wasp *Polistes dominulus* (Hymenoptera: Vespidae; Polistinae). Journal of the New York Entomological Society **108(3-4):** 314-325.

Post D. C., Downing H. A., Jeanne R. L. (1984) Alarm response to venom by social wasps *Polistes exclamans* and *P. fuscatus* (Hymenoptera, Vespidae). Journal of Chemical Ecology **10:** 1425-1433.

Queller D. C., Zacchi F., Cervo R., Turillazzi S., Henshaw M. T., Santorelli L. A., Strassmann J. E. (2000) Unrelated helpers in a social insect. Nature **405:** 784-787.

Rathjen H. (1999) Neue Nachweise von *Polistes dominulus* (Christ, 1791) in Norddeutschland (Hymenoptera, Vespidae). Mitteilungsblatt der Arbeitsgemeinschaft ostwestfälisch-lippischer Entomologen **15:** 38-39.

Reeve H. K. (1991) *Polistes*. Pages 99-148 *in* K. G. Ross and R. W. Mathews, editors. The Social Biology of Wasps. Cornell University Press, Ithaca, London.

Reeve H. K., Starks P. T., Peters J. M., Nonacs P. (2000) Genetic support for the evolutionary theory of reproductive transactions in social wasps. Proceedings of the Royal Society B: Biological Sciences **267:** 75-79.

Richards O. W. (1978) The Australian social wasps (Hymenoptera: Vespidae). Australian Journal of Zoology **61 (Suppl.):** 1-132.

Ross K. G., Matthews R. W. (1991) The Social Biology of Wasps. Cornell University Press, Ithaca, London.

Santos B. F., Payne A., Pickett K. M., Carpenter J. M. (2015) Phylogeny and historical biogeography of the paper wasp genus *Polistes* (Hymenoptera: Vespidae): implications for the overwintering hypothesis of social evolution. Cladistics **31**: 535–549.

Schmaranzer S., Stabentheiner A. (1988) Variability of the thermal behaviour of honeybees on a feeding place. Journal of Comparative Physiology B **158:** 135-141.

Seeley T. D. (1974) Atmospheric Carbon Dioxide Regulation in Honey-Bee *(Apis mellifera)* colonies. Journal of Insect Physiology **20:** 2301-2305.

Seeley T. D. (1997) Honigbienen. Im Mikrokosmos des Bienenstocks. Birkenhäuser-Verlag, Basel, Boston, Berlin

Shakarad M., Gadagkar R. (1995) Colony founding in the primitively eusocial wasp, R*opalidia marginata* (Hymenoptera: Vespidae). Ecological Entomology **20:** 273-282.

Simon Thomas R. T. (1984) *Polistes* (P.) *gallicus* (Linnaeus) nieuw voor de Nederlandse fauna (Hymenoptera: Vespidae). Entomologische Berichten Amsterdam **44:** 113-116.

Sledge M. F., Dani F. R., Fortunato A., Maschwitz U., Clarke S. R., Francescato E., Hashim R., Morgan E. D., Jones G. R., Turillazzi S. (1999) Venom induces alarm behavior in the social wasp *Polybioides raphigastra* (Hymenoptera: Vespidae): an investigation of alarm behavior, venom volatiles and sting autotomy. Physiological Entomology **24:** 234-239.

Stabentheiner A., Schmaranzer S. (1987) Thermographic Determination of Body Temperatures in Honey Bees and Hornets: Calibration and Applications. Thermology **2:** 563-572.

Staines C. L., Smith I. B. (1995) *Polistes dominulus* (Christ) (Hymenoptera: Vespidae) new to Maryland. Proceedings of the Entomological Society of Washington **97:** 891.

Steiner A. (1930) Die Temperaturregulierung im Nest der Feldwespe *(Polistes gallica var. biglumis L.).* Zeitschrift für vergleichende Physiologie **11:** 461-502.

Steiner A. (1932) Die Arbeitsteilung der Feldwespe *Polistes dubia* K. Zeitschrift für vergleichende Physiologie **17:** 101-151.

Tautz J., Maier S., Groh C., Rössler W., Brockmann A. (2003) Behavioural performance in adult honey bees is influenced by the temperature experienced during their pupal development. Proceedings of the National Acadamy of Sciences (PNAS) USA **100:** 7343-7347.

Tautz J. (2007) Phänomen Honigbiene. Spektrum, Elsevier Spektrum akademischer Verlag, München.

Tibbetts E. A., Reeve H. K. (2003) Benefits of foundress associations in the paper wasp *Polistes dominulus*: increased productivity and survival, but no assurance of fitness returns. Behavioral Ecology **14:** 510–514.

Turillazzi S. (1980) Seasonal variation in the size and anatomy of *Polistes gallicus* (L.) (Hymenoptera: Vespidae). Monitore zoologico italiano (N. S.) **14:** 63-75.

Turillazzi S., Marino-Piccioli M. T., Hervatin L., Pardi L. (1982) Reproductive capacity of single foundress and associated foundress females of *Polistes gallicus* (L.) (Hymenoptera Vespidae). Monitore zoologico italiano (N. S.) **16:** 75-88.

Turillazzi S., West-Eberhard M. J. (1996) Natural History and Evolution of Paper-Wasps. Oxford University Press, Oxford.

Weidenmüller A., Kleineidam C., Tautz J. (2002) Collective control of nest climate parameters in bumblebee colonies. Animal Behaviour **63:** 1065-1071.

Weiner S. A., Woods W. A. Jr., Starks P. T. (2009) The energetic costs of stereotyped behaviour in the paper wasp, *Polistes dominulus*. Naturwissenschaften **96:** 297-302.

Weiner S. A., Upton C. T., Noble K., Woods W. A. Jr., Starks P. T. (2010) Thermoregulation in the primitively eusocial paper wasp, *Polistes dominulus*. Insectes sociaux **57:** 157-162.

Weiner S. A., Noble K., Upton C. T., Woods W. A. Jr., Starks P. T. (2011) A role for thermoregulation in the *Polistes dominulus* invasion: a comparison of the thermoregulatory abilities of the invasive wasp *P. dominulus* and the native wasp *P. fuscatus*. Insectes sociaux **58:** 185-190.

Weiner S. A., Galbraith D. A., Adams D. C., Valenzuela N., Noll F. B., Grozinger C. M., Toth A. L. (2013) A survey of DNA methylation across social insect species, life stages, and castes reveals abundant and caste-associated methylation in a primitively social wasp. Naturwissenschaften **100:** 795-799.

Wenzel J. W. (1996) Learning, behavior programs, and higher-level rules in nest construction of *Polistes*. Pages 58-74 *in:* S. Turillazzi and M. J. West-Eberhard, editors. Natural History and Evolution of Paper-Wasps. Oxford University Press, Oxford.

West-Eberhard M. J. (1969) The social Biology of Polistine Wasps. Miscellaneous Publications Museum of Zoology, University of Michigan NO **140:** 1-101.

West-Eberhard M. J. (2006) Polistine passions. Annales Zoologici Fennici **43:** 387-389.

West M. J. (1967) Foundress associations in Polistine wasps: Dominance hierarchies and the evolution of social behaviour. Science **157:** 1584-1585.

Wenzel J. W. (1996) Learning, behavior programs, and higher level rules in nest construction of *Polistes*. Pages 58-74 *in* S. Turillazzi and M. J. West-Eberhard, editors. Natural History and Evolution of Paper-Wasps. Oxford University Press, Oxford.

Weyrauch W. (1936) Das Verhalten sozialer Wespen bei Nestüberhitzung. Zeitschrift für vergleichende Physiologie **23(1):** 51-63.

Willink A., Chiappa E. (1993) Lista de las especies chilenas de la familia Vespidae (Hymenoptera: Vespidae). Acta Entomológica Chilena **18:** 119-125.

Witt R. (2009) Wespen. Vademecum Verlag, Oldenburg, Germany.

Woods W. A., Heinrich B., Stevenson R. D. (2005) Honeybee flight metabolic rate: does it depend upon air temperature? Journal of Experimental Biology **208:** 1161-1173.

Yamane S. (1972) Life Cycle and Nest Architecture of *Polistes* Wasps in the Okushiri Island, Northern Japan (Hymenoptera, Vespidae). Journal oft he faculty of science Hokkaido University series VI Zoology **18 (3):** 440-459.

Yamane S., Kawamichi T. (1975) Bionomic comparison of *Polistes biglumis* (Hymenoptera, Vespidae) at two different localities in Hokkaido, northern Japan, with reference to its probable adaptation to cold climate. Kontyû **43(2):** 214-23.

Yamane S. (1996) Ecological factors influencing the colony cycle of *Polistes* wasps. Pages 75-97 *in* S. Turillazzi and M. J. West-Eberhard, editors. Natural History and Evolution of Paper-Wasps. Oxford University Press, Oxford.

Yamane S., Kudô K., Tajima T., Nihon´yanagi K., Shinoda M., Saito K., Yamamoto H. (1998) Comparison of investment in nest construction by the foundresses of consubgeneric *Polistes* wasps, *P. (Polistes) riparius* and *P. (P.) chinensis* (Hymenoptera, Vespidae). Journal of Ethology **16 (2):** 97-104.

Yamane S., Mateus S., Hozumi S., Kudo K., Zucchi R. (2009) How does a colony of *Apoica flavissima* (Hymenoptera: Vespidae, Epiponini) maintain a constant temperature? Entomological Science **12:** 341-345.

Zanette L., Field J. (2008) Genetic relatedness in early associations of *Polistes dominulus:* from related to unrelated helpers. Molecular Ecology **17:** 2590–2597.

Zanette L., Field J. (2009) Cues, concessions, and inheritance: dominance hierarchies in the paper wasp *Polistes dominulus.* Behavioral Ecology **20:** 773-780.

Zanette L. R. S., Field J. (2011) Founders versus joiners: group formation in the paper wasp *Polistes dominulus.* Animal Behaviour **82:** 699-705.

LIST OF FIGURES

LIST OF TABLES

SUPPLEMENTAL MATERIAL

TABLE S1

Table of newly founded nests within the three years of observation.

Year	Site-no.	Name of the site	Internal no.	Consecutive no.
2012	1	RH (vineyard house)	12.1.1	1
			12.1.2	2
			12.1.3	3
			12.1.4	4
			12.1.5	5
	2	WB (vineyard metal tubes)	12.2.1	6
			12.2.2	7
			12.2.3	8
			12.2.4	9
			12.2.5	10
			12.2.6	11
			12.2.7	12
	3	BS (Beestation barn)	12.3.1	13
	4	EH (Barn in town)	12.4.1	14
			12.4.2	15
			12.4.3	16
			12.4.4	17
			12.4.5	18
			12.4.6	19
	5	ZooIII.1 (Wooden box 1)	12.5.1.1	20
			12.5.1.2	21
			12.5.1.3	22
			12.5.1.4	23
			12.5.1.5	24
			12.5.1.6	25
			12.5.1.7	26
	6	ZooIII.2 (Wooden box 2)	12.5.2.1	27
			12.5.2.2	28
			12.5.2.3	29
2013	1	RH (vineyard house)	13.1.1	30
	2	WB (vineyard metal tubes)	13.2.1	31

Year	**Site-no.**	**Name of the site**	**Internal no.**	**Consecutive no.**
2013	2	WB (vineyard metal tubes)	13.2.2	32
			13.2.3	33
			13.2.4	34
			13.2.5	35
	3	BS (Beestation barn)	13.3.1	36
			13.3.2	37
	4	EH (Barn in town)	13.4.1	38
			13.4.2	39
			13.4.3	40
			13.4.4	41
			13.4.5	42
			13.4.6	43
	5	ZooIII.1 (Wooden box 1)	13.5.1.1	44
	6	ZooIII.2 (Wooden box 2)	13.5.2.1	45
2014	1	RH (vineyard house)	14.1.1	46
	2	WB (vineyard metal tubes)	14.1.2	47
			14.2.1	48
	2 3	WB (vineyard metal tubes) BS (Beestation barn)	14.2.2	49
			14.2.3	50
			14.2.4	51
			14.2.5	52
			14.3.1	53
	3 4	BS (Beestation barn) EH (Barn in town)	14.3.2	54
			14.4.1	55
	5	ZooIII.1 (Wooden box 1)	14.5.1.1	56
	5 6	ZooIII.1 (Wooden box 1) ZooIII.2 (Wooden box 2)	14.5.1.2	57
			14.5.2.1	58
	5	ZooIII.1 (Wooden box 1)	14.6.1	59
	2	WB (vineyard metal tubes)	14.7.1	60
	2 3	WB (vineyard metal tubes) BS (Beestation barn)	14.7.2	61
			14.8.1	62
	3	BS (Beestation barn)	14.8.2	63

TABLE S2

Table of old abandoned nests within four years of observation.

Winter season of extrication	Site no.	Name of site	No. of cells	Consecutive no.
2011/2012	1	RH (vineyard house)	140	1
			129	2
			138	3
	2	WB (vineyard metal tubes)	105	4
			55	5
			141	6
			64	7
	3	WBS (vineyard Sonnenstuhl)	101	8
	4	WG (attic of an old house)	63	9
			40	10
			45	11
			29	12
			38	13
			34	14
			43	15
			81	16
	5	OS (barn within forest)	224	17
			164	18
	2	WB (vineyard metal tubes)	23	19
	6	BS (Beestation barn)	27	20
			11	21
			19	22
			10	23
			18	24
			37	25
			35	26
			16	27
			155	28
			129	29
			199	30
			98	31
			124	32
	1	RH (vineyard house)	33	33
	7	EH (barn in town)	15	34
			22	35
			32	36
			40	37
			39	38
			30	39
			195	40
			68	41
			63	42

Winter season of extrication	Site no.	Name of site	No. of cells	Consecutive no.
2011/2012	7	EH (barn in town)	94	43
			74	44
			176	45
			75	46
			95	47
			190	48
			27	49
			34	50
			23	51
			37	52
			37	53
			54	54
			139	55
			118	56
			141	57
	8,1	ZooIII.1 (wooden box 1)	55	58
			63	59
			54	60
			100	61
			79	62
			147	63
			212	64
			158	65
	8,2	ZooIII.2 (wooden box 2)	229	66
			181	67
			142	68
			135	69
			110	70
			124	71
			71	72
			132	73
			22	74
			12	75
			22	76
			23	77
			40	78
	9	HH (attic of a new house)	117	79
			222	80
			168	81
			163	82
			144	83
			90	84
			145	85
			123	86

Winter season of extrication	Site no.	Name of site	No. of cells	Consecutive no.
2011/2012	9	HH (attic of a new house)	95	87
			98	88
2012/2013	1	RH (vineyard house)	108	89
			120	90
			78	91
			109	92
			19	93
	6	BS (Beestation barn)	16	94
			90	95
	8,1	ZooIII.1 (wooden box 1)	56	96
			17	97
			9	98
			20	99
			11	100
			52	101
			32	102
			16	103
			17	104
	8,2	ZooIII.2 (wooden box 2)	81	105
			52	106
			64	107
			24	108
			35	109
			13	110
	7	EH (barn in town)	113	111
			105	112
			12	113
			27	114
			20	115
			60	116
2013/2014	2	WB (vineyard metal tubes)	89	117
			70	118
			72	119
			75	120
	1	RH (vineyard house)	40	121
	2	WB (vineyard metal tubes)	31	122
			42	123
			31	124
			31	125
			32	126
	6	BS (Beestation barn)	9	127
			58	128
	7	EH (barn in town)	53	129
			34	130

Winter season of extrication	Site no.	Name of site	No. of cells	Consecutive no.
2013/2014	7	EH (barn in town)	11	131
			35	132
			9	133
			56	134
	8,1	ZooIII.1 (wooden box 1)	44	135
	8,2	ZooIII.2 (wooden box 2)	9	136
2014/2015	1	RH (vineyard house)	141	137
			147	138
	2	WB (vineyard metal tubes)	110	139
			108	140
			100	141
			107	142
			130	143
	6	BS (Beestation barn)	111	144
	7	EH (barn in town)	139	145
	8,1	ZooIII.1 (wooden box 1)	150	146
	8,2	ZooIII.2 (wooden box 2)	92	147

ACKNOWLEDGEMENTS

I have had the support and encouragement of many people during the years I have worked on this thesis. With these Acknowledgements, I send my warmest regards to everyone who helped making it possible.

First of all, I would like to thank my thesis committee for fantastic discussions and their support. Without their constant encouragement, this project would not have been possible. I wish to express my sincere gratitude to my thesis supervisor **Prof. Dr. Jürgen Tautz** for providing me the opportunity to work on this very exciting topic, picking me up again and always believing in me. I thank my second supervisor **PD Dr. Johannes Spaethe** for many stimulating discussions which always brought the best out of me and my data. I also thank **Prof. Dr. Stefano Turillazzi**, my third supervisor, for his help, the discussions and especially for his willingness to take over the second report.

I am very grateful to **Prof. Dr. Hildburg Beier,** who has always been there when I needed help and provided her complete experience. Thanks a lot for many hours of constructive discussions, the thorough proofreading of manuscripts and plenty of advice. To the same extent, I am thankful to **Prof. Dr. Hans J. Gross** for his constant interest on this work, his "open ear" for all kind of problems I faced during my PhD time and all the received suggestions and constructive comments.

I would like to thank my current and former colleagues, students and assistants. First of all, many thanks to **Dirk Ahrens.** I enjoyed the experience working with him for years and the great friendship that has developed. I am deeply thankful for the contribution of **Shawn Kennedy** and **Theresa Hügel,** who have done some of the time-consuming analysis of many hours of thermovision-data sets. Thanks to **Dr. Klara Azzami, Dr. Heike Gätschenberger, Leonie Lichtenstein, Frank Sommerlandt, Daniel Materna, Anissa Dudde and Tina Lachowicz** for many talks, which sometimes went beyond science. Furthermore, I would like to thank all other members of the department of Zoology II for the friendly togetherness.

Equally, thanks to my Bachelor students **(Daniel Beck, Oliver Britsch, Natalie Hirtz, Theresa Hügel, Katja Rahn and Cornelia Vogt)** and my trainees **(Sandra Kehrberger, Shawn Kennedy, Ann-Kathrin Kiesel, Richard Lehmann and Stephanie Pinzel),** who made my PhD time very interesting and diverting. Thanks to **Oliver Schätzlein** (president of AKAN e. V.) for his help finding nesting sites in the surrounding of Würzburg.

I also thank my **entire family** and all of **my friends,** who supported me where they could. Special thanks to **Dr. Michael Oleszczuk** for the last-minute proofreading of parts of this thesis.

I wholeheartedly thank my partner **Rolf Dast**, who supported me not only with his love and understanding all the time, but also in finding nesting sites and sometimes with his craftsmanship. Without his help and motivation, this work would never have been completed.

I particularly want to thank my mother **Erika Höcherl,** who has shown to me what it means to be determined and what somebody can reach with a strong force of will. She always believed in me, supported every decision I made, motivated me and last but not least enabled my study. Words alone cannot express my gratitude!

Finally, I would like to thank the "**Graduate School of Life Sciences"** and the "**Qualifikationsprogramm für Wissenschaftlerinnen"** at the University of Würzburg for providing funding through PhD scholarships. In particular, I would like to thank **Dr. Gabriele Blum-Oehler** (GSLS) and **Gisela Kaiser** (Frauenbüro) for their support and help in all questions about my fellowships.

Those that I have inadvertently forgotten to mention are thanked for their help as well as for their understanding and forgiveness.

PUBLICATIONS & CONFERENCE CONTRIBUTIONS

PEER-REVIEWED ARTICLES

Junker R. R., **Höcherl N.,** Blüthgen N. (2010) Responses to olfactory signals reflect network structure of flower-visitor interactions. Journal of Animal Ecology 79: 818-823.

Höcherl N., Siede R., Illies I., Gätschenberger H., Tautz J. (2012) Evaluation of the nutritive value of maize for honey bees. Journal of Insect Physiology 58: 278-285.

Höcherl N., Tautz J. (2015) Thermoregulation of individual paper wasps *(Polistes dominula)* plays an important role in nest defence and dominance battles. Science of Nature 102:32 DOI 10.1007/s00114-015-1283-6.

Höcherl N., Tautz J. (2015) Nesting behavior of the paper wasp *Polistes dominula* in Central Europe – a flexible system for expanding into new areas. Ecosphere 6(12): 262 DOI 10.1890/ES15-00254.1.

Höcherl N., Kennedy S., Tautz J. (2016) Thermoregulation of *Polistes dominula* nests. Modern methods – new insights. (in preparation).

NON-PEER-REVIEWED ARTICLES

Ahrens D., **Höcherl N.** (2010). Bienen sind keine Teetrinker. Schweizerische Bienenzeitung 7: 30-32.

Höcherl N., Illies I., Siede R., Tautz J. (2010). Effects of a pure maize pollen diet on the honeybee. Apidologie 41: 681.

Ahrens D., **Höcherl N.,** Gätschenberger H., Azzami K., Tautz J. (2010). Honeybees are not tea drinkers. Apidologie 41: 687-688.

Ahrens D., **Höcherl N.** (2010). Mit Tee in den Winter? ADIZ/db/IF 7: 22-23.

Höcherl N., Illies I., Tautz J. (2011). Longevity of workers fed with maize pollen. Apidologie 42: 783.

Höcherl N., Siede R., Illies I., Gätschenberger H., Tautz J. (2012). Immer nur Maispollen? ADIZ/db/IF 5: 10-13.

Hirtz N., Ahrens D., Gätschenberger H., **Höcherl N.,** Pahl M., Tautz J. (2012). For Drones the serious side of life begins at day six. Apidologie AGIB 2012 Abstracts: 25.

Vogt C., **Höcherl N.,** Härtel S. (2012). A colony based drone keeping method allows analysis of sperm characteristics in honey bee drones. Apidologie AGIB 2012 Abstracts: 15.

CONFERENCE CONTRIBUTIONS

Höcherl N., Illies I., Siede R., Tautz J. (2010). Auswirkungen einer einseitigen Maispollenernährung auf die Honigbiene *(Apis mellifera* L.*)*. 57. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung, Bochum, Germany (Presentation)

Ahrens D., **Höcherl N.,** Gätschenberger H., Azzami K., Tautz J. (2010). Bienen sind keine Teetrinker. 57. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung, Bochum, Germany (Poster)

Höcherl N., Illies I., Tautz J. (2011). Untersuchungen zur Langlebigkeit von Arbeiterinnen unter einseitiger Maispollenernährung. 58. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung, Berlin, Germany (Poster)

Hirtz N., Ahrens D., Gätschenberger H**., Höcherl N.,** Pahl M., Tautz J. (2012). An Tag sechs beginnt für Drohnen der Ernst des Lebens. 59. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung, Bonn, Germany (Poster)

Hügel T., **Höcherl N.,** Härtel S. (2012). Combined effects of Bt-maize and *Nosema ceranae* on longevity and gut function of honey bee workers. 59. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung, Bonn, Germany (Poster)

Rahn K., **Höcherl N.,** Härtel S. (2012). Does Bt-pollen affect the flight behavior and mortality of honey bee workers under colony conditions? 59. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung, Bonn, Germany (Poster)

Vogt C., **Höcherl N.,** Härtel S. (2012). A colony based drone keeping method allows analysis of sperm characteristics in honey bee drones. 59. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung, Bonn, Germany (Poster)

Höcherl N., Tautz J. (2012). Thermoregulation of *Polistes dominulus*-Nests. EPOS, Würzburg, Germany (Poster)

Höcherl N., Tautz J. (2013). Regulieren primitiv eusoziale Wespen die Temperatur ihrer Nester? 60. Jahrestagung der Arbeitsgemeinschaft der Institute für Bienenforschung, Würzburg, Germany (Poster)

Höcherl N., Kennedy S., Tautz J. (2013). Smoke causes „Hot Bees“. SCIX, Würzburg, Germany (Poster)