# Effects of altitude and canopy cover on the nest size and colony size of the red wood ants *Formica lugubris* and *Formica paralugubris*

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# Abstract

Variations in life-history characteristics across geographic gradients may have implications for the impact of environmental change on animals. Linking one of the most important life-history characteristics and a geographic gradient, Bergmann's rule describes body size increase with increasing latitude. Due to comparable thermal patterns between latitude and altitude, a similar process is expected to apply across altitude. For social insects, the colony could be biologically analogous to the body of a unitary organism. This study investigates the relationship between altitude and colony size in social insects. The model species used were wood ants *Formica lugubris* and *F. paralugubris*. These species have a flexible nesting strategy known as polydomy. I therefore considered both nest size and colony size. Initially, I developed an accurate mark-release-recapture method to estimate nest size, and found that mound volume can be a useful nest size index. A detailed case-study focused on canopy cover effects and showed that nests were larger in shadier areas. Informed by the results, I finally assessed the relationship between altitude, canopy cover, polydomy, nest size and colony size. The results reveal that colony size follows Bergmann's rule along altitude when canopy cover is controlled for: microclimatic factors can be more significant than geographic factors in determining colony size. A systematic review in the Appendix shows that *F. lugubris* populations in different locations differ in mean nest size, but shows no evidence of a trade-off between nest size and multi-nest organisation. This thesis not only provides the first intra-specific evidence of Bergmann's rule acting at the colony level across altitude, but also indicates the prominent role of microclimate on a key life-history characteristic. The work therefore sheds light on the evolution of an eco-geographic cline and the effects which climate change may have on the cline.

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# Declaration

I hereby declare that this submission is entirely my own work except where due acknowledgement is given.

Chapter 2 has been published in *Insectes Sociaux* (Chen & Robinson, 2013) and is presented as published.

Chapters 4 has been published in *PLoS ONE* (Chen & Robinson, 2014) and is presented as published.

Chapter 5 is currently being written into a manuscript for submission.

# Chapter 1 – General Introduction

The world that we live in obeys fundamental laws of physics. Certain elements, materials and energy react and interact with each other according to their properties: for example, the rate (time) of gas diffusion depends on the nature of other gases or solvents and the temperatures (energy) in given spaces (Philibert, 2005). All biological phenomena or activities of an organism are also bound by the control of these laws of physics. For example, most enzymes, which affect the life of organisms, are active within a relatively small range of temperatures (Suzuki, 2015). Based on these laws, body size of an organism determines its surface-area-to-volume ratio, and then subsequently constrains and shapes the biological characteristics of the organism. Taking the exchange of materials between the body and the environments for example, more complex and efficient mechanisms of oxygen transport should be developed with increasing body size, otherwise oxygen cannot reach every cell efficiently (Calder, 1996).

## Body Size

In the first chapter of *Size, Function and Life History*, Calder (1996) states: "Suppose we encounter a new beast……if we know only its weight, we can predict a wide variety of its specifications and requirements: home range, heart and metabolic rate and life span - each from an empirical allometric equation based on body size." There is probably no doubt that body size is one of the most fundamental characteristics of any animal because it is associated with most important aspects of an animal's biology (Calder, 1996; Brown & Lomolino, 1998; Chown & Gaston, 2010). Taking metabolic rate for example: the relationship between body size and metabolic rate persists in a 3/4-power scaling, even though body size can range across 21 orders of magnitude from the smallest microbes to the largest mammals (Fig. 1.1, adapted from West *et al.*, 2000). The metabolic transformation contributes both energy and the materials to run all biological functions and build all organismal structures, so metabolic rate constrains all biological activities at all organisation levels, from molecules, cells to individuals and populations (West *et al.*, 2000). Body size therefore can be considered the most relevant and requisite factor for quantitative analyses of patterns in the comparative physiology and life history of animals (Calder, 1996).

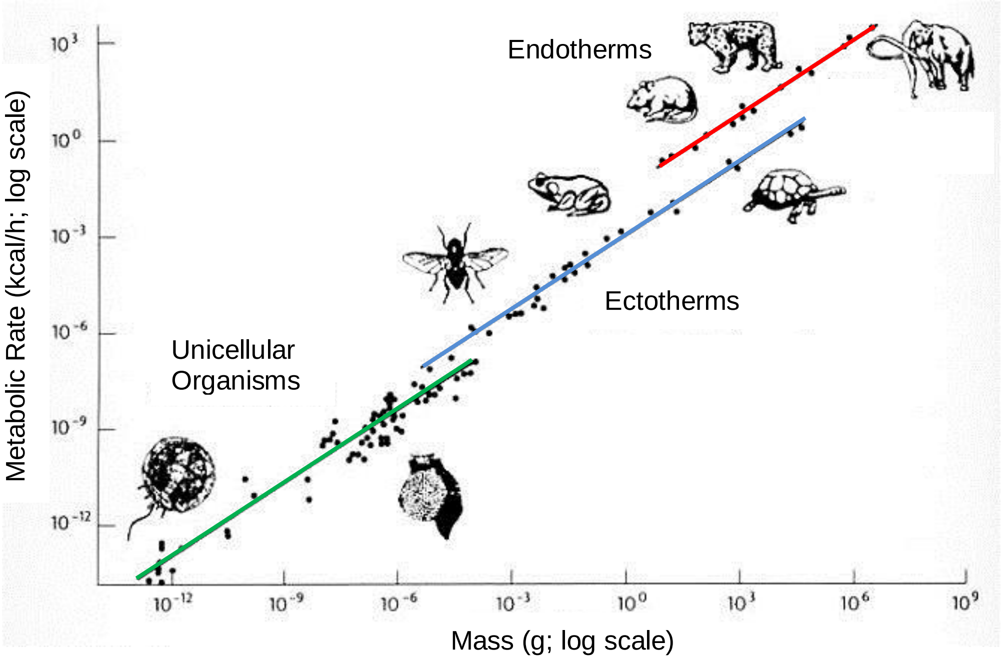

**Figure 1.1.** The relationship between metabolic rate and body mass for a series of organisms ranging from the smallest microbes (green line), ectotherms (blue line) to the largest endothermic mammals (red line) (adapted from West *et al.*, 2000).

There is barely any law that can be applied universally, and “rules” are usually more flexible than “laws” (Lawton, 1999; Watt & Salewski, 2011). The Oxford Dictionary defines law as “a statement of fact, deduced from observation, to the effect that a particular natural or scientific phenomenon always occurs if certain conditions are present”, for example, the three laws of thermodynamics. Ecological experiments cannot be easily replicated due to the constantly changing background environments, animal behaviours and ecosystem states (Knapp *et al.*, 2004). Therefore laws are too restrictive for ecology, instead, rules are proposed in ecology: “a rule reflects the notion of generality and conditional probability, but places less restrictive boundaries on expectations” (Watt *et al.*, 2010). There are several ecological rules which are all descriptions of patterns (Mayr, 1956). These rules are empirical generalisations and independent of mechanisms (Meiri, 2011). Some of these ecological rules regard animals’ body sizes. Cope's rule describes the tendency for lineages to increase in body size over evolutionary time (Rensch, 1948; Hone & Benton, 2005). Foster's rule (also known as the island rule) is a tendency stating that small mammals evolve larger size, whereas large mammals evolve smaller size on islands (Lomolino, 1985; Meiri *et al.*, 2008).

The “bigger is better” rule proposes that, within a population, individuals with larger body size tend to have greater performance and fitness than those with smaller size (Kingsolver & Huey, 2008). Large body size brings some obvious fitness benefits (reviewed by Dibattista *et al.*, 2007), for example, larger individuals may have (1) access to more types of food, (2) increased competitive ability, (3) better endurance of severe conditions or diseases, (4) earlier maturation and (5) higher reproductive output. According to the fitness benefits of larger body size, it seems that the “bigger is better” rule should predict a directional selection favouring increased size.

However, growing to and maintaining a larger size also involves costs and risks: for example, to achieve a large body size requires a long developmental stage, which may negatively affect fecundity and survival, and thus is negative to fitness (Kingsolver & Huey, 2008). Therefore the "bigger is better" rule will be true, given that the positive effects of larger body size overcome the negative effects of longer developmental time on fitness. Other negative effects of larger body size may also include greater resource requirements (McNab, 2010).

Based on the concept of fitness optimisation, life history theory involves the specific strategies used by organisms to cope with their environments (Stearns, 1992; Vuarin *et al.*, 2012). Biologists therefore are curious about the relationships among environmental factors, organismal lifestyes and life-history characteristics. Another rule involves the relationship between the prominent characteristic, body size, and a large-scale environmental factor: Bergmann claims a body size change across the geographic gradient, latitude (Bergmann, 1847; Watt *et al.*, 2010).

## Bergmann's Rule

Bergmann's rule (Bergmann, 1847) is one of the oldest and most studied eco-geographic rules of body size. James (1970) provided a translated excerpt of this rule: "…it is obvious that on the whole the larger species live farther north and the smaller ones farther south." According to the excerpts translated by James (1970), Bergmann's rule originally has linked temperature to latitude because temperature on a global scale decreases from the equator to the poles. The decline of temperatures with rising altitude and latitude is the main comparable similarity between altitude and latitude (Brown & Lomolino, 1998). The same relationship along latitude might be applied to altitudinal gradients. Watt *et al.* (2010) also gave a translation of Bergmann's rule: "if there would be genera, which species are distinguished as much as possible only by size, the smaller species would all need a warmer climate."

However, the ranges of body size vary among species: should we study an ecological pattern with the control of phylogenetic constraints even within a genus? The issue of whether Bergmann's rule should be applied inter- or intra-specifically has been discussed for a long time. On the one hand, Bergmann originally proposed to apply his rule inter-specifically (within genera) to homeotherms (endotherms) (James, 1970; Watt *et al.*, 2010). The redefinition of the rule by Blackburn *et al.* (1999) also supported inter-specific studies: "the tendency for a positive association between the body mass of species in a monophyletic higher taxon (e.g. species within genera or within families) and the latitude inhabited by those species." On the other hand, a suggestion was made by James (1970) that intra-specific and inter-specific geographic patterns of body size variation should be separately formalised. James intends that the extension of Bergmann's rule within species could be defined as the neo-Bergmann's rule. Angilletta *et al.* (2004a) also states that Bergmann's rule could be used to describe the pattern of a species' body size change along latitude (intra-specifically). Furthermore, Bergmann attempts to test his rule among races of domestic animals (within species) (Watt *et al.*, 2010). More than 160 years after Bergmann's study, recently, Watt *et al.* (2010) review the original definition and several suggested redefinitions of Bergmann's rule (Table 1.1). Meiri (2011) suggests that the rule is a pattern that can be studied at any taxonomic level and in any taxon. Bergmann's rule is used more loosely to describe a trend of the body size increases with rising latitude or decline of temperature either intra-specifically or inter-specifically.

**Table 1.1.** Original definition and suggested redefinitions of Bergmann’s rule from the review of Watt *et al.* (2010).

| | | | | |
|---|---|---|---|---|
| Bergmann’s rule | Inter-specific | Endothermic | Temperature | Bergmann (1847) |
| | | | Latitude | Bergmann (1847);<br>Blackburn *et al.* (1999) |
| | | Ectothermic | Temperature | |
| | | | Latitude | Blackburn *et al.* (1999) |
| | Intra-specific | Endothermic | Temperature | Rensch (1938); James (1970); Paterson (1990) |
| | | | Latitude | |
| | | Ectothermic | Temperature | Paterson (1990) |
| | | | Latitude | |

It has been established for a long time that Bergmann’s rule is applicable to endotherms in terms of ecology and morphology (Mayr, 1963). More than 65% of endotherms show Bergmann’s cline in body size (i.e. body size increases with rising latitude) (Ashton *et al.*, 2000; Ashton, 2002; Meiri & Dayan, 2003). The fasting endurance hypothesis (also known as starvation resistance) states that more seasonal environments favour larger body size because larger animals can store more fat and then survive during seasonal stress (Lindstedt & Boyce, 1985; Millar & Hickling, 1990). The mass of body fat scales as total body mass to greater than the 1.0 power for both birds and mammals (Lindstedt & Boyce, 1985; Calder, 1996): larger endotherms have both relatively and absolutely greater energy stores. Furthermore, as mentioned before, larger animals have a lower metabolic rate per weight. Greater energy stores and lower weight-specific metabolic rates therefore benefit larger endotherms via fasting endurance.

The suggested revisions of Bergmann’s rule by Paterson (1990) and Blackburn *et al.* (1999) do not exclude ectotherms (reviewed by Watt *et al.* (2010), Table 1.1). A review study by Vinarski (2014) shows that there is not a universal pattern in the geographic variation of body size for each large taxon of ectotherms (e.g. molluscs, arthropods, amphibians, reptiles, etc.). Vinarski (2014) claims that it is still not feasible to judge the occurrence of Bergmann's cline in ectotherms, because of the low number of studies of these taxa. In the phylum Arthropoda, Bergmann's rule has been best investigated for the class Insecta (Diptera, Lepidoptera, Neuroptera, Homoptera, Hymenoptera, etc) (Table 1.2, adapted from Shelomi, 2012; Vinarski, 2014). In this relatively well-studied group, both Bergmann's cline (body size increases with rising latitude) and converse Bergmann's cline (body size decreases with rising latitude) were found in species belonging to different orders (Shelomi, 2012). Two different aspects of hypothesised mechanisms are suggested to apply Bergmann's rule to ectotherms.

**Table 1.2.** Number of studies in insects with Bergmann's, converse-Bergmann's, and no clines, compared inter- and intra-specifically within the type of range examined (latitude or altitude) (adapted from Shelomi, 2012).

| | Inter-specific | Intra-specific | Total |
|---|---|---|---|
| Latitude: | | | |
| Bergmann's cline | 18 | 123 | 141 |
| Converse-Bergmann's cline | 12 | 111 | 123 |
| No cline | 36 | 114 | 150 |
| Altitude: | | | |
| Bergmann's cline | 6 | 75 | 81 |
| Converse-Bergmann's cline | 15 | 98 | 113 |
| No cline | 21 | 150 | 171 |

Both adaptive and non-adaptive hypotheses arise to explain Bergmann's cline. The non-adaptive hypothesis illustrates how thermal effects on biochemical processes can result in the temperature-size relationship (Angilletta *et al.*, 2004b). Originally, Ray (1960) proposed a relationship between body size and temperature named "temperature-size rule". It describes that a smaller final body size of ectotherms is produced at increased rearing temperature under laboratory conditions (Atkinson, 1994; Angilletta & Dunham, 2003). This thermal plasticity in body size is a taxonomically widespread "rule" in biology because it has been reported for bacteria, protists, plants and ectotherms (Angilletta *et al.*, 2004b). This rule is met in more than 84% of all ectotherms (Atkinson, 1994). Atkinson (1994) suggested three key thermal effects to understanding temperature-size relationships: thermal constraints on maximal body size, thermal sensitivities of growth rate, and thermal sensitivities of juvenile survivorship.

From the other perspective, the adaptive hypothesis considers the costs and benefits of a given life history to describe the reason that natural selection promotes genotypes which grow more slowly but mature at a larger size when raised at lower temperatures (Atkinson & Sibly, 1997; Angilletta *et al.*, 2004b). Taking some insect and reptile species for example, this can be achieved by the delay of maturation in their life cycle: the transition from a one-generation-per-year cycle to a one-generation-per-two-years cycle at high latitudes results in an increase in the final body size (reviewed by Vinarski, 2014). Although the increasing body size seems to be a by-product of delayed maturation and not an independent adaptation, larger body size indeed benefits ectotherms with the greater fasting endurance ability (Cushman *et al.*, 1993; Blackburn *et al.*, 1999; Vinarski, 2014). The adaptive hypothesis states that the body size variation in Bergmann's rule should be based on geographically-based genetic differences, so thermal plasticity (differences in growth rate and developmental rate at different temperatures) (van der Have & de Jong, 1996) is not the underlying mechanism.

Vinarski (2014) provides an opinion that the adaptive and non-adaptive hypotheses cannot always be separated distinctly: “Any mechanism increasing fitness may be regarded as adaptive in a certain sense”. Thermal plasticity in body size represented as the temperature-size rule can be considered as a kind of “adaptive” characteristic in some aspects. A larger body size may result from a longer duration of growth or faster growth, or both. However, ectotherms grow more slowly at lower temperatures. A larger body size in cold environments should be achieved by delayed maturation for prolonged growth (Atkinson, 1994, and references therein; Angilletta *et al.*, 2004a). In addition, it is also possible that Bergmann’s clines are formed as a result of the decrease in size at low latitudes (high temperatures) rather than the increase at high latitude. The solubility of oxygen in water decreases with increasing temperature. The limitation on oxygen may reduce the body size of aquatic and some terrestrial ectotherms (Atkinson, 1994).

According to the temperature-size rule for ectotherms, if the body size is mainly determined by temperature, Bergmann’s clines would have been observed in those ectotherms with wide distribution ranges. However, the review by Vinarski (2014) has shown that there is not a single universal pattern of body size change for ectotherms. The temperature-size rule arose based on the results of laboratory experiments with other environmental factors controlled. Under natural conditions, it is very likely that these environmental factors (e.g. moisture content and primary production) overcome or form synergistic interactions with the impact of thermal effects on an organism (Vinarski, 2014).

## Microclimate

In addition to the large-scale geographic gradients, small-scale environmental factors also contribute to local physical conditions of the environment. Microclimate is the set of climatic conditions measured in local areas near the ground surface (Chen *et al.*, 1999; Geiger *et al.*, 2009). This set of measurements includes temperature, light, wind speed, and moisture. Although studies of eco-geographic rules focus on the large-scale ecological questions, there are some reasons that these studies should consider or include the concept of microclimate. Firstly, relationships between microclimates and ecological processes are ubiquitous and complex, for example, the limitations on local light, temperature, moisture and vapour pressure may constrain the plant distributions (Chen *et al.*, 1999). Secondly, by adjusting local distribution or changing their use of different habitats, animals can still find appropriate microclimates under the impact of the large-scale environmental conditions (Suggitt *et al.*, 2012). Changes driven by microclimate in habitat use can shape or shift species' distribution dynamics and their responses to environmental change (Lawson *et al.*, 2014). Moreover, microclimate may also be associated with animal's body size (Cagle *et al.*, 1993; Kaspari, 1993; Dawson *et al.*, 2005). For these reasons, microclimate effects should be considered in studies of any eco-geographic rule.

For terrestrial ecosystems, habitat type (e.g. desert, grassland and woodland) is a major modifier of the microclimate experienced by organisms, for example, it affects the extreme value of temperatures (Suggitt *et al.*, 2011). Taking woodland habitat as an example, microclimatic variables, especially solar radiation, local air temperature (air temperature at the ground surface) and soil temperature, are highly sensitive to the canopy variation between sites (Chen & Franklin, 1997). Small canopy openings are a common and significant cause of woodland spatial heterogeneity (Clinton, 2003). Canopy features contribute to structural complexity and provide high spatial and temporal variability on the forest floor within woodland habitat (Chen & Franklin, 1997; Chen *et al.*, 1999). Canopy cover therefore could be a feasible and practical index of microclimate for woodland habitat.

## The Social Insect Colony

Social insects have two levels of organisation, the individual and the colony. The allometry or biological scaling should be important at both organisation levels. On the one hand, body size, which influences all aspects of biology, is one of the most significant characteristics of an individual. On the other hand, individuals of social insects form a new level of organisation that has its own biological or physiological properties, by living together and coordinating their collective activities. According to the concept "Insect Sociometry" proposed by Tschinkel (1991), the colonies can also be characterised by their physical and numerical features. The biomass of a colony, the total number of individuals (including brood), or the worker population in a colony has been used to represent the size of a colony (Kaspari & Vargo, 1995; Tschinkel, 1998, 1999).

Selection may act at the level of the colony when it favours the evolution of traits that reinforce the survival and reproduction of a colony (Crozier & Consul, 1976; Frumhoff & Ward, 1992). The colony of social insect, for several reasons, can be considered to be the biological analogue of the body of an individual organism (Tschinkel, 1991; Kaspari & Vargo, 1995; Tschinkel, 1998, 1999; Clémencet & Doums, 2007; Lanan *et al.*, 2011). Firstly, just as body size plays a prominent role for a unitary organism, colony size is associated with many biological aspects of a social insect colony (Dornhaus *et al.*, 2012). Colony size is correlated to competitive abilities in ants (Palmer, 2004); brood number in bees (Eckert *et al.*, 1994); foraging behaviours in wasps (O'Donnell & Jeanne, 1992), bees (Eckert *et al.*, 1994) and ants (Herbers & Choiniere, 1996); colony-level organisation in ants (Holbrook *et al.*, 2011; Schmidt *et al.*, 2011; Dornhaus *et al.*, 2012); lifespan in wasps (O'Donnell & Jeanne, 1992); and thermoregulation ability in ants (Rosengren *et al.*, 1987).

Secondly, individuals of a social insect colony and cells of a unitary organisms show similarities in terms of organisation. A multicellular individual can also be considered as a “complex society” of cells, just as a colony can be thought of as a complex society of individuals (Anderson & McShea, 2001). Anderson and McShea (2001) illustrate the similar relationship between complexity and aggregate size (body size of a multicellular individual or colony size of a social insect colony) which are generally applying to complex societies of cells of multicellular organisms and to colonies of multicellular individuals: the complexity of the society increases with aggregate size increasing. One of the indices of complexity is degree of differentiation. Larger systems are expected to be more differentiated: larger body size with more cell types, larger colony size with higher worker specialisation. Another similarity of organisation between unitary organisms and social insect colonies is shown by the allometry of reproduction. Larger unitary organism species invest proportionately less in their offspring (Reiss, 1991). Per-capita productivity, defined as the ratio of total new workers and sexuals produced to the total number of adult workers, usually decreases in larger colonies (reviewed by Kramer *et al.*, 2014).

Finally, metabolic scaling theory and empirical studies also show that individuals of a colony function similarly to cells of a multicellular organism in physiological features. Several features of physiology (e.g. metabolic rate and gonad tissue mass) and life history (e.g. growth and lifespan) of the whole social insect colony also follow the same size-dependencies as unitary organisms when a colony’s summed biomass is analogous to the mass of individuals (Gillooly *et al.*, 2010; Hou *et al.*, 2010). For example, the slope for the relationship between colony biomass and metabolic rate is statistically indistinguishable from the predicted value of a 3/4-power scaling as seen in the unitary animals (Fig. 1.1, West *et al.*, 2000; Hou *et al.*, 2010). In addition, the difference of metabolic rate between individual bees and bees in a colony is similar to the difference between cells in vitro and cells in vivo (Fig. 1.2, adapted from Gillooly *et al.*, 2010). A hypothesis is proposed that the mechanism limiting the exchange rate of energy and materials is the same in both whole social insect colonies and unitary organisms (Hou *et al.*, 2010). Thus, the colony size could be analogous to the body size of a social insect colony.

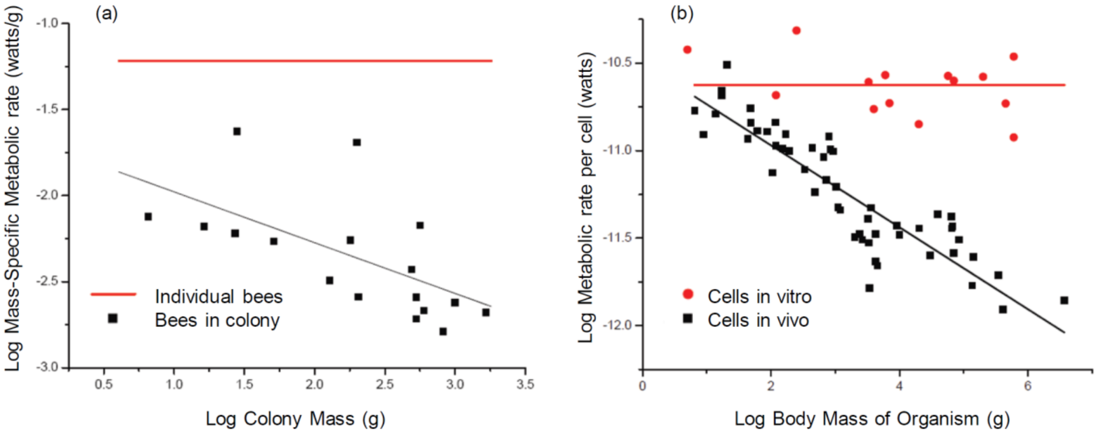

**Figure 1.2.** Comparison of the metabolic rate changes between individual bees and bees in a colony (a), and between cells in vitro and cells in vivo (b). Data for individual bees is averaged from two references (adapted from Gillooly *et al.*, 2010).

## Red Wood Ants and Polydomy

The red wood ant group (also known as *Formica rufa* group) comprises six morphologically similar species in temperate and boreal forests across northern Eurasia (Cotti, 1996; Bernasconi *et al.*, 2011). These ants, which are considered keystone species, are ecologically dominant and have impacts at multiple community levels including ants, other arthropods and vertebrates (Sudd & Lodhi, 1981; Savolainen & Vepsäläinen, 1988; Haemig, 1992; Punttila *et al.*, 1994; Rolstad *et al.*, 2000; Punttila *et al.*, 2004; Kilpeläinen *et al.*, 2005). Red wood ants may also function as ecological indicators in European broadleaf forest and taiga, e.g. for climate warming and land-use changes by changes in their distribution and abundance (Ellison, 2012). Wood ants defend large territories against other ant species (Savolainen & Vepsäläinen, 1988, 1989) and, as predators, hunt on trees and on the ground in woodlands (Sudd & Lodhi, 1981). Their main food resource is honeydew excreted from the tended aphids, which are also a source of protein for these ant species (Rosengren & Sundström, 1991). By tending sap-sucking aphids, the ants can also affect tree growth (Rosengren & Sundström, 1991). Maintaining long-lasting foraging trails between nest and trees, on which they tend aphids, is associated with the stability of food resource.

Red wood ants are known for their large and long-lived nests, which are rich in organic material (Ohashi *et al.*, 2007; Domisch *et al.*, 2008). They build nests with both underground (chambers) and aboveground (mounds) parts. The large mineral-soil mound part is built on the soil surface and covered by forest litter, such as pine needles, twigs, resin, and bark to form thatch parts (Laakso & Setälä, 1998; Ohashi *et al.*, 2007). In addition to metabolic heat produced by individual ants, microbial decomposition of these organic materials functions as another heat source (Frouz, 2000). Wood ants also accumulate nutrients in the mounds, for example, carbon and nutrient concentrations are apparently higher in their mounds than the surrounding forest floor (Laakso & Setälä, 1998; Lenoir *et al.*, 2001). Their mounds can act as habitats for myrmecophiles and influence the nutrient cycle of the forest (Laakso & Setälä, 1997, 1998; Domisch *et al.*, 2008; Jurgensen *et al.*, 2008; Robinson & Robinson, 2013). Because of their strong impact on forest ecosystems (Laakso & Setälä, 1997; Ohashi *et al.*, 2007; Żmihorski, 2010) and their "near threatened" situation listed by the International Union for Conservation of Nature (IUCN, 2014), the red wood ant group are protected in many European countries (Bernasconi *et al.*, 2011).

Some wood ant species have a flexible nesting strategy named polydomy: one colony may either build one nest or comprise several spatially separated but socially connected nests (Hölldobler & Wilson, 1977; Ellis & Robinson, 2014; Robinson, 2014). Debout *et al.* (2007) define polydomy as "an arrangement of an ant colony in at least two spatially separated nests", and the detached distance between these two nests should be greater than of the distance between two nest chambers in the core nest structure. Any structure that contains workers and brood is considered as a nest, but the presence of a queen is not an elementary criterion for a nest (Debout *et al.*, 2007). The nest is therefore an extra level of organisation between the individual and the colony. Polydomy has evolved several times in ants, and is found in all the main subfamilies and on all continents where ants occur (Debout *et al.*, 2007).

Potential ecological benefits of being polydomous include risk spreading (Debout *et al.*, 2007; Robinson, 2014) and both resource discovery (Cook *et al.*, 2013) and exploitation (by establishing new nests near food resource) (Lanan *et al.*, 2011). A polydomous colony may also overcome the constraints on increasing size for a monodomous colony (Robinson, 2014), for example, increasing total colony size through increasing nest number rather than increasing individual number of a single nest.

The “domy” forms (whether monodomous or polydomous) vary markedly both within and between red wood ant species. *Formica aquilonia*, *F. lugubris* and *F. polyctena* have been recorded as polydomous in some areas but monodomous in others, however, *F. rufa* and *F. pratensis* are recorded only as monodomous (a colony has only one nest) (Ellis & Robinson, 2014). In wood ants, domy form may also link to reproductive strategy: polydomy is associated with polygyny (multiple queens in a nest), and monodomous colonies are usually monogynous (one queen in a nest) (reviewed by Ellis & Robinson, 2014). In other ants, half of recorded polydomous species are monogynous (Debout *et al.*, 2007). Polydomy is also a factor that must be considered when the study focuses on colony size because of its effects on colony-level organisation.

The chosen species in this study were two sibling and sympatric species *Formica lugubris* and *Formica paralugubris* (Hymenoptera: Formicidae) (Seifert, 1996). These two species are highly similar to each other in aspects of morphology and ecology, and have to be morphologically discriminated from each other under a stereo-microscope (Seifert, 1996). Among the ecologically important red wood ant group, *F. lugubris* is of interest for altitudinal studies because it has been recorded at altitudes from 800 to 2400 metres in Central Europe (Kutter, 1965; cited by Sudd *et al.*, 1977). In the relatively few studies that cover it, *F. paralugubris* also has been found at altitudes from 1100 to 2000 metres (Bernasconi *et al.*, 2006; Glaser, 2006). *Formica lugubris* uses both monodomous and polydomous strategies: monodomous in the Swiss Jura Mountains and polydomous in Great Britain and the Swiss Alps;

whereas *F. paralugubris* has only a polydomous form (Bernasconi *et al.*, 2005; Maeder *et al.*, 2005; Ellis & Robinson, 2014). In the following chapters of this study, a polydomous colony is defined as a group of nests which are connected to each other by trails, which were defined as a distinct path with at least 10 workers in 40 cm (Ellis *et al.*, 2014).

## Rationale for the Thesis and Aims

A comparable similarity between altitude and latitude is the decline of temperatures with rising altitude and latitude (Brown & Lomolino, 1998). If the tendency of body size increase across latitude is driven by temperature, the same relationship along latitude might be applied to altitudinal gradients. The term “altitude” is the vertical distance between an object and a reference point (McVicar & Körner, 2013). In this thesis, altitude is used to indicate the vertical distance from sea level.

If a social insect colony can be biologically analogous to the body of a unitary organism, the colony size can be considered as the “body size” of a colony. This study aims to test whether Bergmann's rule, originally observed between latitude and body size, can be applied to the relationship between altitude and ant colony size (Fig. 1.3). There are some studies on inter-specific or intra-specific Bergmann’s rule between latitude (or altitude) and body size (or colony size) of social insects, but there is a knowledge gap concerning intra-specific studies for Bergmann’s rule between altitude and colony size (Table 1.3).

In terms of altitude, the most obvious and important small-scale factor which is associated with this geographic gradient is the patterns of vegetation. For woodland, the habitat type occupied by red wood ants, one of the indices of patterns of vegetation is canopy cover. Canopy cover is known to be related to ant’s nest size (Ellis *et al.*, 2014). Since temperature, the most likely main driving force of Bergmann’s rule, is also related to canopy cover (Rodriguez-Garcia *et al.*, 2011; Suggitt *et al.*, 2011; Huang *et al.*, 2014), we also included the effect of canopy cover together with altitudinal effects in the investigation of colony size.

**Table 1.3.** Summary of previous research on Bergmann’s rule in social insects, broken down into inter-specific or intra-specific Bergmann’s rule between latitude (or altitude) and body size (or colony size) of social insects. The boxes marked “?” lack data and are the focus of this study. “None found” indicates that no supporting research was found in the given category. Reversed relationship (converse Bergmann’s cline) between latitude (or altitude) and body size (or colony size) were not found in inter-specific nor intra-specific studies. Note this table is reproduced in Chapter 5 as Table 5.1.

| | | | | Support | No relationship |
|---|---|---|---|---|---|
| Bergmann’s rule | Inter-specific | Latitude | Body size | [1][2] | [3] |
| | | | Colony size | [4] | [2][3][5] |
| | | Altitude | Body size | None found | [3] |
| | | | Colony size | None found | [3] |
| | Intra-specific | Latitude | Body size | [6][7][8] | None found |
| | | | Colony size | None found | [8] |
| | | Altitude | Body size | [8][a][9][b] | None found |
| | | | Colony size | ? | ? |

[1] Cushman *et al.* (1993); [2] Kaspari (2005); [3] Geraghty *et al.* (2007); [4] Kaspari and Vargo (1995); [5] Porter and Hawkins (2001); [6] Daly *et al.* (1991); [7] Rust (2006); [8] Heinze *et al.* (2003); [9] Stone (1993); [a] two high altitudinal populations in the study were larger than expected from latitude; [b] a study of solitary bees.

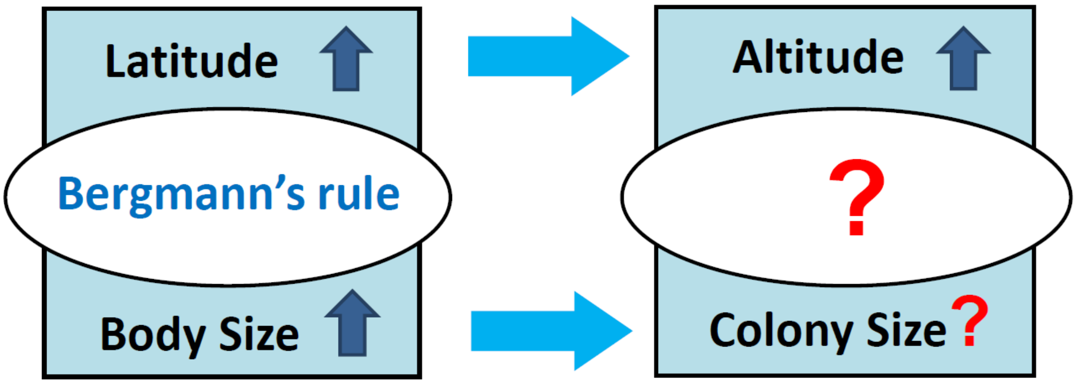

**Figure 1.3.** Aim of this study: to investigate the application of Bergmann's rule to the relationship between altitude and ant colony size.

Establishing appropriate methods to estimate colony size (or nest size) is the first step of this study. In Chapter 2, an accurate mark-release-recapture method was developed. Mound volume was also demonstrated to be a useful nest size index. In Chapter 3, a preliminary test then was conducted to test the feasibility of developed methods to detect altitudinal effects on nest/colony size, and to estimate required sample size for further study. The results of this test also showed that canopy cover (an index of microclimate) may influence nest size. In Chapter 4, I describe a detailed case-study which specifically focused on canopy cover effects on nest size. Temperature and food resource availability were considered as mediating factors underlying the relationship between canopy cover and nest size. Building on the findings of these investigations, a study in Chapter 5 was conducted to determine the relationships between altitude (a geographic gradient), canopy cover, polydomy, nest size and colony size (Fig. 1.3). In Chapter 6, in addition to discussing the limitations of and potential future work arising from this study, a systematic review was conducted from previous studies and found that *F. lugubris* populations in different locations differ in average nest size, but show no evidence of a trade-off between nest size and multi-nest organisation.

# Chapter 2 – A comparison of mark-release-recapture methods for estimating colony size in the wood ant *Formica lugubris*

## Abstract

Colony size can be considered the analogue of the body size of a superorganism. Just as body size is important to the physiology of an individual animal, colony size correlates with the life history and ecology of social insects. Although nest excavation and counting all individuals is the most accurate method for estimating colony size (or nest size), it has the major drawback of being destructive. Alternatively, mark-release-recapture (MRR) can be used repeatedly to measure the size of the same colony or nest. We compared the accuracy and feasibility of four MRR methods and a mound volume method with complete counts from nest excavation for estimating the nest size of *F. lugubris*, a mound-building wood ant of the *Formica rufa* group, during the early spring in Scotland. We found that our After-Disturbing method, in which we performed marking and recapturing after gentle disturbance to the top of nest mound, has the best balance between accuracy, non-destructiveness, and time required. We also found that mound volume can be an index of ant nest size under certain conditions. Both non-destructive methods can be used on the same colony or nest repeatedly to monitor nest dynamics.

# Introduction

Body size is one of the most significant characteristics of an animal because it influences virtually all physiological characters (Brown & Lomolino, 1998; Blanckenhorn & Demont, 2004). In social insects, the colony can be considered the biological analogue of the "body" of an individual organism (Tschinkel, 1991; Kaspari & Vargo, 1995; Tschinkel, 1998, 1999; Clémencet & Doums, 2007; Lanan *et al.*, 2011). As a group of cells are categorized as an organism when the cells build a cooperative unit to reproduce their genes, it could be correct to classify a group of organisms as a superorganism when the organisms construct a cooperative unit to reproduce their genes (Seeley, 1989). Hence, the individual and the superorganism are two levels of organisation of social insects. Studying the "body size" of the colony may reveal how this life-history trait of the superorganism correlates with their lifestyle and habitat. For example, number of workers may be related directly to competitive abilities in ants (Palmer, 2004), foraging behaviour in ants and bees (Eckert *et al.*, 1994; Herbers & Choiniere, 1996), and worker life span of wasps (O'Donnell & Jeanne, 1992; Pamilo *et al.*, 1992). Moreover, it is especially interesting that the size of these superorganisms can have a wide range, for example, over eight orders of magnitude in the ant family Formicidae alone (Kaspari & Vargo, 1995). In addition, in polydomous ants, one colony may settle in either one nest or several spatially separated but socially connected nests (Hölldobler & Wilson, 1977). Although the nests and other structures of ant colonies can be regarded as extensions of the superorganism, Debout *et al.* (2007) suggested that nest-level allocation is subjected to stronger selection than is colony-level allocation in some polydomous ants. Banschbach and Herbers (1996) indicated that only nest-level traits play a major role in determining variation in fitness. Therefore, estimating colony size, as well as nest size of polydomous colonies, is important to understanding the life-history and ecology of social insects.

The total number of individuals or the worker population in a colony (or a nest) has been used to represent the colony size (or nest size) of social insects (Kaspari & Vargo, 1995; Tschinkel, 1998, 1999). In ants, the most accurate colony size estimation method is nest excavation (Elmes, 1974; Gordon, 1992; Tschinkel, 1993; Akre *et al.*, 1994; Beshers & Traniello, 1994; Tschinkel *et al.*, 1995). Although the nest excavation method can obtain the exact count and biomass of all stages in the nest, it is destructive and laborious (Stradling, 1970; Skórka *et al.*, 2006). An additional step has also been used to decrease labour of excavation method in some studies. After excavation and mixing the whole nest soil and ants, the number of individuals in a given sampled mound soil volume was counted, then the nest size was estimated by this number and the total mound volume (Tschinkel, 1993; Tschinkel *et al.*, 1995).

Alternatively, mark-release-recapture (MRR) methods (or capture-mark-recapture, CMR) can monitor the colony dynamics without destroying it (Chew, 1959; Kruk-de Bruin *et al.*, 1977; Breen, 1979; Sundström, 1995; Billick, 1999; Brown *et al.*, 2002; Rosset & Chapuisat, 2007). In general the MRR method is based on some assumptions (Chew, 1959; Stradling, 1970): (1) every individual in the colony is able to be captured and marked; (2) a sample which represents the population of the colony is taken to mark and estimate; (3) the marks are permanent during the sampling period, and the marked individuals are not influenced by them; (4) the marked animals mix thoroughly with unmarked individuals before resampling; (5) the population is closed, the rates of immigration and emigration are known and no births and deaths occur during the period of mixing. From these assumptions, probably the most challenging step of the MRR method is how to ensure all individuals are available to be captured and marked. For example, in carpenter ants, about 80 per cent of the individuals do not forage, so marking on foraging trails can only estimate the forager group (Ayre, 1962). It can also be a problem to capture certain special types of workers such as repletes which usually stay in the deep parts of the nests (Chew, 1959). Then there is a trade-off between the assumption four and the assumptions three and five. To make sure marks are retained and the population is isolated needs a short experimental period. On the other hand, the period should be long enough for marked and unmarked ants to mix thoroughly. Several studies have investigated the feasibility of using non-destructive MRR methods to estimate real parameters of the colony, such as colony size and colony biomass (Table 2.1). Porter and Jorgensen (1980) and Kruk-de Bruin *et al.* (1977) showed that although the estimation from MRR method on foragers (on the trail) only represented the size of the foragers group, it can be used to estimate the whole colony size of *Pogonomyrmex owyheei* and *Formica polyctena* because foragers compose a certain proportion of colony. Besides the forager group, defender estimation was also a good index of colony size in *P. owyheei* (Porter & Jorgensen, 1980). Billick (1999) tested whether the MRR method provided an accurate estimate of worker number of *F. neorufibarbis* by capturing and marking workers after overturning rocks on the nests. Although the fit of the regression line showed by R square value from the studies of foragers is higher (Kruk-de Bruin *et al.*, 1977; Porter & Jorgensen, 1980), the results underestimated the colony size. On the other hand, although the regression line in Billick's study has a lower fit compared to former studies, estimation from the number of workers rather than only of foragers seems to more realistically predict the colony size.

**Table 2.1.** Summary of the results in studies which compared the estimates by non-destructive methods with real parameters of ant colony size.

| Estimates | Real parameters | $r^2$ | n | species | References |
|---|---|---|---|---|---|
| Mound volume | Colony biomass | 0.90 | 30 | *Solenopsis invicta* | (Tschinkel *et al.*, 1995) |
| Nest volume | Total number | 0.87 | 16 | *Formica pallidefulva* | (Mikheyev & Tschinkel, 2004) |
| Defender number (MRR) | Total adults | 0.86 | 12 | *Pogonomyrmex owyheei* | (Porter & Jorgensen, 1980) |
| Forager number (MRR) | Total number | 0.86 | 15 | *F. polyctena* | (Kruk-de Bruin *et al.*, 1977) |
| Mound volume | Colony biomass | 0.85 | 75 | *S. invicta* | (Tschinkel, 1993) |
| Removed worker number# | Worker number | 0.83 | 21 | *Myrmica ruginodis* | (Skórka *et al.*, 2006) |
| Forager number (MRR) | Total adults | 0.81 | 10 | *P. owyheei* | (Porter & Jorgensen, 1980) |
| Territory area | Worker biomass | 0.80 | 30 | *S. invicta* | (Tschinkel *et al.*, 1995) |
| Basal area of mound | Worker number | 0.79 | 30 | *F. podzolica* | (Savolainen *et al.*, 1996) |
| Territory area | Colony biomass | 0.79 | 30 | *S. invicta* | (Tschinkel *et al.*, 1995) |
| Worker number (MRR) | Worker number | 0.77 | 6 | *F. neorufibarbis* | (Billick, 1999) |
| Territory area | Worker number | 0.76 | 30 | *S. invicta* | (Tschinkel *et al.*, 1995) |
| Removed worker number# | Worker number | 0.69 | 76 | *M. scabrinodis* | (Skórka *et al.*, 2006) |
| Removed worker number# | Worker number | 0.66 | 27 | *M. rubra* | (Skórka *et al.*, 2006) |
| Depth of the nest | Worker number | 0.61 | 24 | *Cataglyphis cursor* | (Clémencet & Doums, 2007) |
| Surface area of the nest dome | Worker number | 0.59 | 59 | *F. exsecta* | (Liautard *et al.*, 2003) |
| Surface area of the nest dome | Brood production | 0.55 | 59 | *F. exsecta* | (Liautard *et al.*, 2003) |
| Basal area of nest | Alate mass | 0.25 - 0.52 | 49 | *F. podzolica* | (Deslippe & Savolainen, 1994) |

#Number of workers removed by sticks in a given period (see text in introduction); $r^2$: r square value; n: sample size

In addition to the above main methods, in some studies the colony size or nest size was related to or estimated by other non-destructive methods using the features of nest, such as the basal area or the volume of nest mound (Deslippe & Savolainen, 1994; Savolainen *et al.*, 1996; Liautard *et al.*, 2003; Sorvari & Hakkarainen, 2007; Sorvari, 2009). Table 2.1 shows several studies which compared the nest features with the real parameters of colony. Tschinkel (1993) and Tschinkel *et al.* (1995) suggest that mound volume of the nest may be a convenient and non-destructive method to estimate the colony biomass of *Solenopsis invicta*. Ground-level area of the mound was related to alate mass (Deslippe & Savolainen, 1994), and worker number (Savolainen *et al.*, 1996). Surface area of the nest dome could predict both worker number and brood production (Liautard *et al.*, 2003). Depth of the nest was also related to colony size (Clémencet & Doums, 2007). However, there are also problems with these methods of estimation. Domisch *et al.* (2008) argued that decomposition could be either increased or decreased by the activity of colony or external reasons such as temperature. Nests of *F. lugubris* and *F. polyctena* in shaded areas have higher mounds (Sudd *et al.*, 1977; Mabelis, 1979). Breen (1979) also indicated that nest diameter was not a useful predictor for the forager population of *F. lugubris*.

Activities of workers or the colony was also measured to estimate colony size in some studies (Table 2.1). Skórka *et al.* (2006) conducted a new method for three *Myrmica* species in which they opened the topmost part of the nest until the first chambers with larvae was found and used sticks to remove workers which climbed up the stick. The number of workers removed in a given period was positively correlated with the number of workers in the nest. The amount of traffic on trails was combined with the number of trails radiating from a nest to estimate the relative size of a nest population of *F. polyctena* (Mabelis, 1979). A similar idea has been used to predict worker numbers of wasps by Malham *et al.* (1991), who counted the number of individuals entering or leaving the colony in a given period. This seemed like a simplified MRR method which omitted the recapturing procedure. The error of the estimated size from this method was high, so it may needed repeating several times on different days for a more accurate average number (Skórka *et al.*, 2006). Tschinkel *et al.* (1995) found that territory area was related to the biomass of worker and colony, and the number of workers, but it would be time consuming to use this index for estimation of colony size.

Several species of ants in the *Formica rufa* group (red wood ants) are considered “near threatened” by the International Union for Conservation of Nature and Natural Resources (IUCN, 2014) and are protected by law in many European countries (Bernasconi *et al.*, 2011) because of their strong impact on forest ecosystems (Laakso & Setälä, 1997; Ohashi *et al.*, 2007; Żmihorski, 2010). Complete excavation of nests is therefore not feasible as a routine method for studies which need to estimate wood ant colony size or nest size. We compared the feasibility and the accuracy of several MRR methods and the nest mound volume for estimating the nest size of red wood ants. To seek out the best balance for the five assumptions of MRR method, we applied four methods with different levels of invasiveness and collected recapture data over multiple days.

# Materials and Methods

## Species and Location

The choice of model species was *Formica lugubris*, which belongs to the well-studied *Formica rufa* group in Europe (Cotti, 1996). *Formica lugubris* has both monodomous and polydomous social forms (Bernasconi *et al.*, 2005; Maeder *et al.*, 2005), and is polydomous in Great Britain (Sudd *et al.*, 1977). The experiment was conducted in Inshriach forest in the Cairngorms National Park of Scotland in April 2012. Temperatures ranged from 3 to 11$^{o}$C. An area of the forest, approximately 25 hectares, planted primarily with Canadian lodgepole pine (*Pinus contorta*) was to be clear felled in summer 2012 in order to restore native woodland flora, so colonies of *F. lugubris* in this area were to be severely disrupted. This made the site appropriate for applying invasive measures to the wood ant nests. A preliminary survey recorded 24 nests in approximately three hectares along the forest edge of this area and no *F. lugubris* nests in an approximately eight-hectare deep-forest area. No other species of wood ants were present. To test our nest size estimation method, we selected 15 nests that provided a wide distribution of nest sizes and were accessible for excavation. The minimum distance between these nests and neighbouring nests was greater than 15 metres.

## Methods

We applied four MRR methods and a mound volume estimation method to the nests. For our four MRR methods, we marked ants on Day 0, and counted the ratio of marked and unmarked ants on Day 1 and Day 2. For each nest, ants were marked by one person with Pactra® paints (Testors, USA) applied as a dot on the gaster using match sticks in three of the methods and with Brillo® spray leather dye

(Moneysworth & Best, Canada) in the fourth. Different colours of paints were used for the four MRR methods for each nest. The colours used for these methods were varied between colonies. Pactra paint has been used to mark many ant species in previous studies (Fewell, 1990; Fewell *et al.*, 1992; Brown & Gordon, 1997; Haight, 2012). Laboratory preliminary tests established that both Pactra paint and spray dye can be retained on the cuticle of *F. lugubris* workers for more than two weeks and do not contribute to ant mortality over this time period.

### *On-the-Trail Method*

On Day 0, we used Pactra paint to mark and count foragers passing in either direction along the strongest foraging trail at a distance of 0.3-1 meters from the nest, for 15 minutes. On Day 1 and Day 2, the numbers of outgoing foragers marked and unmarked individuals were counted and recorded by one person along the same trail for 15 minutes. We counted only outgoing foragers to avoid recounting the same foragers if they left and returned to the nest in a short period.

### *On-the-Surface Method*

A different colour of Pactra paint was used to mark workers directly on the nest surface for 15 minutes, regardless of whether the ants had already been marked by the first colour. The number of marked workers was recorded. Each day for the next two days, we did recapturing work on the nest surface using a single visual scan sample, which means that one person scanned the whole nest surface only once to count the numbers of marked (with the relevant colour) and unmarked (not painted with that colour) workers.

### *After-Disturbing Method*

To make more workers emerge from the nest, we disturbed the nest by lightly tapping the top of the nest by hand for 5 seconds. We then marked workers directly on the surface with a third colour of paint for 15 minutes, regardless of whether they had already been marked by another colour/s. On Day 1 and Day 2, we disturbed the nest in the same way and then counted the number of marked (with relevant colour) and unmarked workers on the nest surface using a single visual scan sample by one person.

### *Mound-Sampling Method*

Nests were categorised by approximate mound size (small: < 20L, medium: 20-85L, and large: > 85L). On Day 0, an appropriate volume (0.5L, 2L or 4L for small, medium or large nest mound respectively) of mound thatch containing workers was collected from the south-facing part of nest mound and placed in a small bin. We marked all workers in the bin with spray leather dye, regardless of whether they had already been marked by any Pactra paint, but without obscuring other paint marks, and returned all collected soil and ants to the mound. On Day 1, we collected the same volume of thatch to count the spray-marked and unmarked ants then returned all thatch and ants. The same procedures were conducted on Day 2.

### *Mound-Volume Method*

To estimate mound volume, the longest basal diameter, the perpendicular diameter and the height of the nest mounds were measured. If a nest was settled on the slope, uphill height of the nest was used as the height of the nest. Relative mound volume was calculated by multiplying these three dimensions.

### *Nest Excavation*

As far as possible, we completed all four MRR methods in the order listed above for each nest, however we could not complete all four MRR methods for certain nests, especially the On-the-Trail method, because of the absence of ants on trails due to cold weather. We chose 11 of the original 15 nests according to the completeness of our data for each nest and aiming to maintain a wide distribution of nest size. We excavated these 11 nests and counted the actual number of workers. We first removed the thatch of the above ground parts and counted the workers within the nest material, then dug out the underground chambers of the nest, counting the ants in the soil. We used 12-volt car batteries to drive 35-watt car vacuums and aspirators for collecting and counting ants individually. After excavating the nests and counting the actual number of ants, we relocated the ants with their nest material out of the area which will be clear felled.

## Statistical Analyses

For each of our four MRR methods, the estimated nest size was calculated using Bailey's (1951) unbiased modified formula, which is thought to have a better estimate than Lincoln index when marked number is small (Stradling, 1970; Gaskell & George, 1972; Paulson & Akre, 1991): $N = T^*(n + 1) / (t + 1)$, where $N$ is the estimated total number of workers in the nest, $T$ is the number of marked ants, $n$ is the total number in the recapture sample (marked and unmarked workers), and $t$ is the number of marked workers in the recapture sample. We excluded data for which the total number in the recapture sample ($n$) was smaller than total marked ants ($T$) in our MRR methods. Due to the temperature limitations on foraging, the On-the-Trail data were available on only one day and from only 7 nests. For each of the other

MRR methods, we estimated the nest size from the Day 1 and Day 2 data separately, and also took the mean of estimated nest size from the two recapture days for analysis. For each nest, if data from one of the days were excluded because the total recapture sample number was too small, data from the other day was used as the mean. We used simple linear regression for the relationship between the estimated nest size and the actual nest size. Bayesian information criterion (BIC) (Schwarz, 1978) and adjusted $r^2$ were used for comparison and measuring how well a model performs (Seber & Lee, 2003; Fox, 2008; Bingham & Fry, 2010). BIC is considered more appropriate than Akaike information criterion (AIC) (Akaike, 1974) if the sample size is larger than 7 (Seber & Lee, 2003; Fox, 2008), though these two methods gave very similar results when applied to our regression models. All data were transformed by $\log_{10}$ to normalise the distributions, and regressions were conducted with the JMP statistics package (version 6.0.0; SAS institute, Cary, NC, USA).

## Results

We found that the After-Disturbing method, in which ants were marked on the nest surface after mild disturbance, was the best MRR method for predicting the actual nest size of *F. lugubris*. Both the estimates of nest size from the mean of two days' data and from the Day 2 data significantly predicted the actual nest size, with the mean estimated nest size a particularly good predictor (Table 2.2; Fig. 2.1a,c). In addition to the After-Disturbing method, the estimated nest size from the Day 1 data of On-the-Surface method (Table 2.2; Fig. 2.1e) and the Day 2 data of Mound-Sampling method (Table 2.2; Fig. 2.1i) also significantly predicted the actual nest size. As for other methods, the Mound-Volume method weakly predicted the actual nest size with a borderline significant relationship between relative mound volume and actual nest size ($P$ = 0.054, Table 2.2; Fig. 2.2). There was no significant relationship between the actual nest size and the estimated nest size from the On-the-Trail method (Table 2.2).

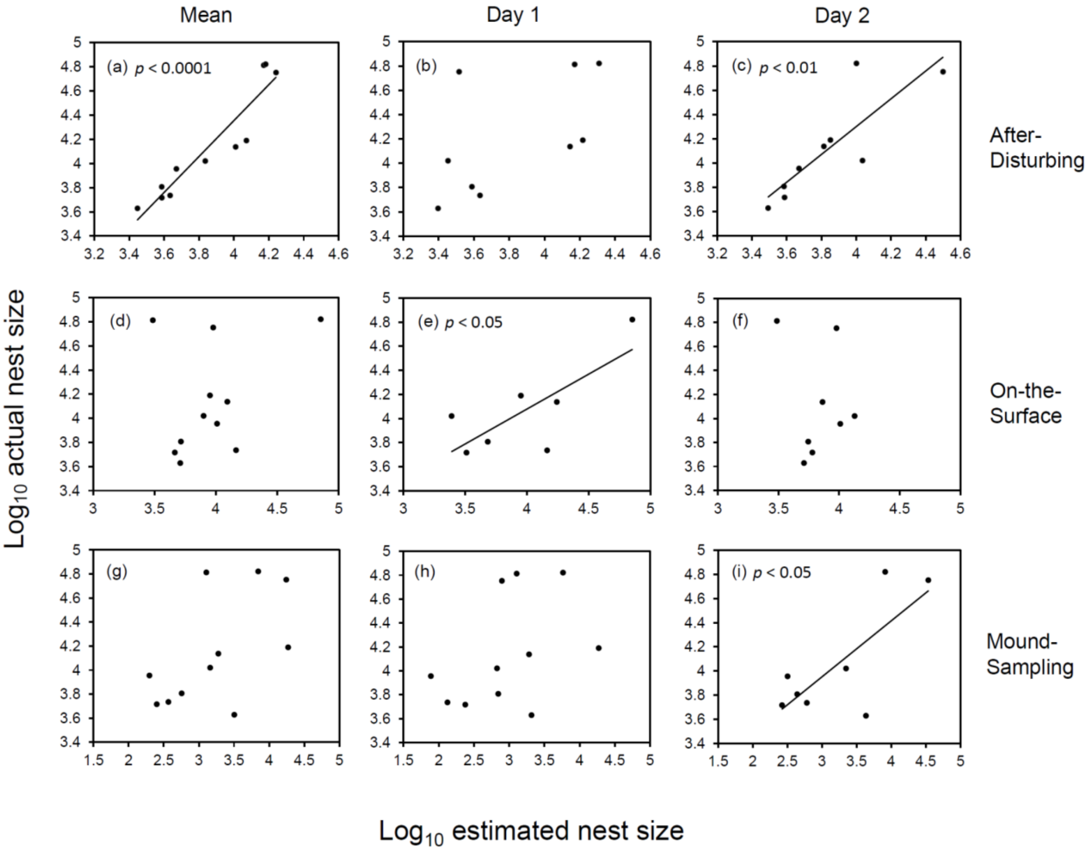

**Figure 2.1.** The relationship between actual nest size and estimated nest size from three mark-release-recapture methods, presenting estimates from Days 1 and 2 of recapture, and also the mean of these estimates (a-c: After-Disturbing, d-f: On-the-Surface, g-i: Mound-Sampling; Regression lines show the significant relationships).

**Table 2.2.** Results of the linear regression for the relationships between the estimated colony size and the actual colony size from five methods.

| Method | Day | n | *F ratio* | *P* | Relationship | $r^2$ | $r^2_{adj}$ | BIC |
|---|---|---|---|---|---|---|---|---|
| On-the-Trail | 1 | 7 | 0.73 | 0.43 | - | - | - | - |
| On-the-Surface | Mean | 11 | 1.14 | 0.31 | - | - | - | - |
| | 1 | 7 | 6.71 | $< 0.05$ | $\log_{10} y = 0.5796 \times \log_{10} x + 1.7594$ | 0.57 | 0.49 | -16.48 |
| | 2 | 8 | 0.14 | 0.72 | - | - | - | - |
| After-Disturbing | Mean | 11 | 66.38 | $< 0.001$ | $\log_{10} y = 1.4874 \times \log_{10} x - 1.5951$ | 0.88 | 0.87 | -37.03 |
| | 1 | 9 | 3.39 | 0.11 | - | - | - | - |
| | 2 | 9 | 17.17 | $< 0.01$ | $\log_{10} y = 1.1448 \times \log_{10} x - 0.2789$ | 0.71 | 0.67 | -23.27 |
| Mound-Sampling | Mean | 11 | 4.57 | 0.06 | - | - | - | - |
| | 1 | 11 | 1.93 | 0.20 | - | - | - | - |
| | 2 | 8 | 7.91 | $< 0.05$ | $\log_{10} y = 0.4644 \times \log_{10} x + 2.5583$ | 0.57 | 0.50 | -18.98 |
| Mound-Volume | - | 11 | 4.91 | 0.05 | $\log_{10} y = 0.5846 \times \log_{10} x + 1.1512$ | 0.35 | 0.28 | -18.43 |

y: actual colony size, x: estimated colony size; n: sample size; $r^2_{adj}$: adjusted r square; BIC: Bayesian information criterion

The actual nest size of these 11 nests ranged from 4,251 to 66,285 ants (Mean ± SD = 23442 ± 25518). Average percentage of marked workers in recapturing day of four MRR methods ranged from 1.6% to 11.7% (Table 2.3). We recorded 3, 7 and 18 queens in 3 of 11 nests. We were not aiming specifically to record queens, and it is likely that more queens were present in these and the other nests, but were not identified due to the quick counting of large numbers of individual ants. Many larvae and eggs were found in the biggest and third biggest nests respectively. Larger workers, which are repletes with distended gasters, were usually found in the underground part of the nests. Few workers were found in the chambers in the north-facing part of the nest thatch. Nests extended approximately 0.5 m deep underground.

Ants marked by spray dye and paints were found deep in the underground parts of nest. No mortality of marked ants was observed during the experiment nor in the dumping places of nests.

**Table 2.3.** Average numbers of marked and recaptured workers for our four MRR methods (Mean ± SD).

| | | | Day 1 | | | Day 2 | | |
|---|---|---|---|---|---|---|---|---|
| Methods | Marked number | n | Recaptured number | % of marked number | n | Recaptured number | % of marked number | n |
| On-the-Trail | 93 ± 41 | 7 | 116 ± 66 | 1.6 ± 0.9 | 7 | | | |
| On-the-Surface | 200 ± 89 | 11 | 621 ± 37 | 3.1 ± 2.7 | 7 | 455 ± 217 | 2.3 ± 1.0 | 8 |
| After-Disturbing | 196 ± 69 | 11 | 579 ± 404 | 3.0 ± 2.2 | 9 | 475 ± 275 | 3.0 ± 1.9 | 9 |
| Mound-Sampling | 110 ± 112 | 11 | 327 ± 242 | 11.7 ± 9.5 | 11 | 247 ± 208 | 6.0 ± 6.5 | 8 |

n: sample size

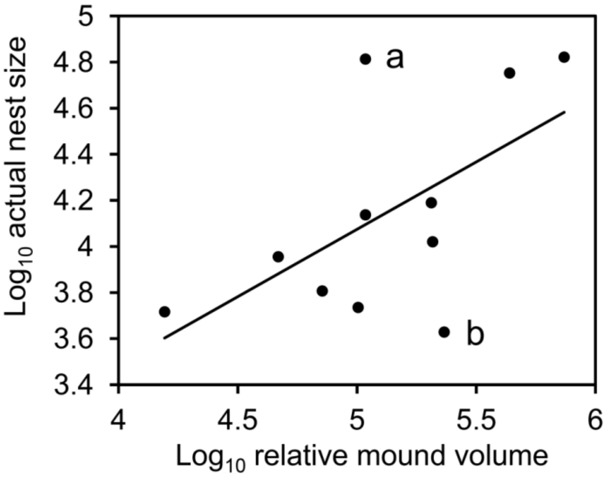

**Figure 2.2.** The relationship between actual nest size and the relative mound volume. Letters (a and b) indicate two nests with atypical mound shape (see text for discussion).

# Discussion

We tested four mark-release-recapture methods involving different levels of disturbance, for estimating nest size and also investigated the relationship between nest mound size and nest size of *Formica lugubris*. We found that our "After-Disturbing" method, in which we performed marking and recapturing after lightly tapping the nest mound surface, was the best method to estimate nest size. Estimates of nest size from this method effectively predict nest sizes ranging from 4,000 to over 60,000 workers. Estimates from the mean of two days recapture data contributed to a better fitting regression model than using either day alone, however, estimates from Day 2 data alone may provide the best balance between accuracy and effort, not only because they require less recapture effort but also because the regression model makes a more realistic estimate: In the model of relationship between actual nest size and the estimated nest size from After-Disturbing method in Day 2, parameters of the equation slope and intercept made the regression line the closest to the equation $y = x$, thus with the least underestimating comparing to other methods (Fig. 2.1).

Lightly tapping the nest mound surface in the After-Disturbing method was probably an important step. Compared to the On-the-Surface method in which workers are not disturbed, adding this step meant that not only can more workers in total be marked and recaptured but also probably includes workers from other task groups switching to nest defence. Thus, adding this step increases the accuracy of the assumptions, that a representative sample of the colony is taken to mark and estimate, improving the prediction of nest size. In contrast, the On-the-Trail and On-the-Surface methods may only capture certain groups so the estimates from these methods are less likely to represent the whole nest. The same situation may occur in the Mound-Sampling method, in which we only sampled part of the mound. Individuals do not distribute evenly in red wood ant nests (Coenen-Stass *et al.*, 1980), so sampling position within the mound may greatly influence the representativeness of the sampling. Even by lightly disturbing, as we did in After-Disturbing and Mound-Sampling methods, we still cannot capture and recapture all types of workers in a nest. It is likely that this is why all estimates from our four MRR methods underestimated the nest size. However, we found a balance between increased accuracy and reduced destructiveness and time for estimating wood ants nest size, as well as the colony size of monodomous species.

MRR methods require that paint marks are permanent over the time-scale of the study. In laboratory preliminary tests we found that both Pactra Paint and Brillo spray dye can be retained on the workers for more than two weeks and do not influence survival. In the Mound-Sampling method, if the ants we captured had been marked by other paint, we sprayed Brillo dye carefully to keep the former mark still visible. Bright and obvious colours such as yellow and pink were used in our experiments and were detected easily. We observed some painted individuals when we were excavating the underground parts of the nests. This sheds light on the assumption of thorough mixing of marked and unmarked workers, suggesting that ants do move through different parts of the nest. Although Porter and Jorgensen (1980) proposed that recapture should be done within one day for the minimum effects of high forager mortality in harvester ants, this does not seem to apply to *F. lugubris* and our MRR methods. The significant relationships between actual and estimated nest size of After-Disturbing and Mound-Sampling method from Day 2 data showed that a period of two days provided a better estimate, compared to one day, suggesting that waiting the extra day allowed colonies to mix more thoroughly.

As for the assumption of closed population, our study was conducted during mid-April, at which point there are no pupae and no eclosions in *F. lugubris* (Cherix *et al.*, 2006). The turnover rate of workers was probably low at this period due to the extended longevity caused by winter of the present workers (Calabi & Porter, 1989). The duration of the experiment was also not long enough to cause a considerable change of worker number from births and deaths. Furthermore, although *F. lugubris* is polydomous in Great Britain (Sudd *et al.*, 1977), we chose nests with at least a 15 metre distance and without obvious trails between them. Low activity caused by cold weather in April helps to meet the assumption of closed population.

Some previous studies have shown that physical features of the nest such as nest volume, mound volume and basal area of mound can be used to predict the colony size or colony biomass of ants (Table 2.1) (Tschinkel, 1993; Tschinkel *et al.*, 1995; Savolainen *et al.*, 1996; Mikheyev & Tschinkel, 2004), however, other studies found contrasting results (Sudd *et al.*, 1977; Breen, 1979; Domisch *et al.*, 2008). In our study, Mound-Volume method provided a borderline significant relationship between relative mound volume and actual nest size. Looking into the details of the mound dimensions we found that two outliers in Fig. 2.2 (points a and b) were from nests with atypical mound shape. Point (a) was from a nest which built the mound on a steep slope. This may have caused us to underestimate the height of the mound. In contrast, a nest which built the mound partly on a fallen tree contributed the data of point (b). This may have caused us to overestimate its height. The relationship between relative mound size and actual nest size would be significant if these two data points were excluded ($F = 19.30$, $P < 0.01$, n = 9, $r^2 = 0.73$). In addition, our study was conducted at the beginning of spring, before *F. lugubris* starts to modify the mound (Cherix *et al.*, 2006). Mound volume would be expected to be most stable during this period, when the building work of the previous summer and the decomposition of the previous autumn and winter have had time to stabilise. Therefore, although some studies showed that mound features should not be used as a predictors for the colony size of *F. lugubris* (Sudd *et al.*, 1977; Domisch *et al.*, 2008), we believe that measuring Mound-Volume method can be a feasible wood ant nest size estimation method, at least in the beginning of spring and for nests with relatively typical mound shape. For the nests settled in a slope, the downhill height of the mound is probable a better measurement to calculate relative mound volume rather than the uphill height of the mound.

This study was conducted in April, before colonies became fully active. This posed difficulties for the On-the-Trail method due to cold weather. On the other hand, this season is suitable for the On-the-Surface, After-Disturbing, Mound-Sampling and Mound-Volume methods because the low foraging activity contributes to the isolation of nests and ensures most of the population will be in the nest. It also increased stability of nest population and mound volume compared to other seasons. Because estimates may vary across different seasons due to variation in shading and changing predation rate and food availability, caution should be used if comparing nest sizes across different seasons. However, our method can be applied to the same nest in the same season year by year to monitor the growth or dynamics of nest size. Our After-Disturbing method is probably feasible for many mound-building ant species which would become aggressive and assemble on the mound surface after disturbance of the nest mound. Really counting the exact number of marked and unmarked workers in recapturing may improve the accuracy of our After-Disturbing method. However, the techniques we used, for example, marking individuals on the mound surface and counting by a single scan sample in the recapturing step, can already contribute to an accurate and fast predictor of ant nest size.

To summarize, we found that our After-Disturbing method, in which marking and recapturing were performed after gently disturbance to the nest, provides a feasible mark-release-recapture method to estimate colony size for monodomous mound-building ants, or nest size of polydomous species. We improved the method's compliance with the assumptions of MRR by lightly tapping the mound surface before marking and capturing, and conducting the experiment in the beginning of spring. We found that our After-Disturbing method has the best balance between accuracy, non-destructiveness, and time required. We also found that mound volume can be an index of ant nest size under certain conditions. Both methods can be used on the same nest repeatedly to follow the nest dynamics.

## Acknowledgements

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# Chapter 3 – Preliminary tests and power analyses for the relationship between altitude and ant colony size

## Abstract

Bergmann's rule describes the decline of body size, which is probably the most important characteristic of an animal, from polar to tropical latitudes. This latitudinal relationship can be applied to altitude due to comparable changes of thermal patterns across these two geographic gradients. In social insects, the colony size could be biologically analogous to the body size of a unitary organism because it is associated with many physiological and behavioural features of the colony. This preliminary study aimed to apply Bergmann's rule to the relationship between altitude and ant colony size. In addition to the geographic gradients, other factors such as canopy cover and the aspect of nesting slope may influence environmental conditions. I targeted a wood ant species *Formica lugubris* in the Swiss Jura Mountains. This monodomous (one colony has only one nest) population can simplify my question for this preliminary study. I did not find an altitudinal effect on colony size within a small geographic range, instead, I found that canopy cover and the aspect of nesting slope could be important. According to the results, I plan to conduct a future study on both *F. lugubris* and *F. paralugubris* in the Swiss Alps for a larger altitudinal range. Based on the required sample size calculated from power analyses, mound volume will be used to estimate nest size efficiently to obtain the necessary sample size. The importance of canopy cover and local temperatures of nesting location were addressed. New measurements and analyses of both canopy cover and local temperatures will be performed.

# Introduction

Body size is associated with most aspects of biology for individual animals, including physiological performance, morphology and fitness (Brown & Lomolino, 1998; Blanckenhorn & Demont, 2004; Kingsolver & Huey, 2008). Bergmann's rule is an eco-geographic rule of animal body size: the decline of body size from polar to tropical latitudes (Bergmann, 1847; Watt *et al.*, 2010). Because of comparable patterns of change in temperature between latitude and altitude (Brown & Lomolino, 1998), a similar body size change has also been found along altitudinal gradients in some species (revew study: Shelomi, 2012).

In addition to the individual, the colony is a specific level of organisation for social insects. Just as body size is a fundamental characteristic of a unitary organism, colony size is also associated with many aspects of the life history and physiology of a social insect colony. Therefore, the colony size of a social insect colony could be biologically analogous to the body size of a unitary organism (Kaspari & Vargo, 1995; Hölldobler & Wilson, 2009). The total number of individuals or the total biomass in a colony has been used to represent the size of a colony (Kaspari & Vargo, 1995; Tschinkel, 1998, 1999).

The same relationship between body size and latitude could be applied to altitudinal gradients. The colony size can be considered as the body size of the colony for social insects. Combining these ideas, I conducted this preliminary test for the application of Bergmann's rule to the relationship between altitude and colony size. To investigate the relationship, the chosen species is *Formica lugubris*, which can distribute as high as 2100 metres in altitude (Bernasconi *et al.*, 2006). This species belongs to the red wood ants group which have ecologically important impacts on forest ecosystem (Sudd & Lodhi, 1981; Punttila *et al.*, 2004) and has been listed as "near threatened" by the International Union for Conservation of Nature and Natural Resources (IUCN, 2014). Red wood ants are flexible in colony-level organisation: one colony can either build one nest (monodomy) or several spatially separated but socially connected nests (polydomy) (Debout *et al.*, 2007; Ellis & Robinson, 2014). Having different domy forms brings an extra level, the nest, to the organisation for social insects. Therefore this nesting strategy should be considered when the study focuses on the colony size of red wood ants. Fortunately, *F. lugubris* is monodomous in the Swiss Jura Mountains (Bernasconi *et al.*, 2005). Starting the investigation from the monodomous *F. lugubris* population can simplify my question because in this population, nest size also means colony size.

With regard to altitude, some environmental factors may change along this gradient and have effects on colony size. The pattern of vegetation is the most obvious environmental factor which is associated with an altitudinal gradient. Canopy cover is one of the indices of patterns of vegetation for woodland, and is known to be related to ant nest size (Ellis *et al.*, 2014). Another environmental factor which may affect nest size is the aspect of nesting slope, because it may be associated with the sunshine hours a colony receives. I therefore also investigated the effects of canopy cover and the aspects of nesting slope together with altitude on colony size in this study.

As a preliminary test, the aims of this study were: 1) to test three indices of colony size (nest size) – worker population from After-Disturbing method and Mound-Volume method, and total biomass – for choosing the best measurement method according to the balance between accuracy, effort and time spent; 2) to check whether two local environmental factors, canopy cover and the aspect of nesting slope, may have effects on colony size (nest size) along altitudinal gradients; 3) to estimate minimum required sample size through power analyses of the results for future study.

## Materials and Methods

### Species and Location

In the Swiss Jura Mountains, six wood ant species (*F. polyctena*, *F. rufa*, *F. pratensis*, *F. truncorum*, *F. lugubris* and *F. paralugubris*) have overlapping distributions. Among these species, *F. lugubris* and *F. paralugubris* can be distinguished from the other four species in the field, but have to be separated from each other by stereo-microscope. *Formica lugubris* is principally monodomous in the Swiss Jura Mountains (Bernasconi *et al.*, 2005), whereas *F. paralugubris* is highly polydomous. I therefore aimed to find monodomous colonies of *F. lugubris* in the field but may include some nests of *F. paralugubris* due to the temporary lack of trails between nests. Samples were collected for species identification. In the following text of this chapter, nest size can be taken to mean colony size, if is referring to a monodomous *F. lugubris* colony.

Three 2-km-wide transects were identified in the Swiss Jura Mountains (near Yverdon-les-Bains, Vaud, Switzerland; Fig. 3.1) according to previous records of *F. lugubris* and *F. paralugubris* nests (D. Cherix, personal communication). There are two main coniferous tree species in this area: *Abies alba* and *Picea abies*. The altitude of the survey area ranges from 400 to 1500 metres. For scheduling the surveys, each transect was divided into several zones basically by contour line every 200 metres (letters A to O in Fig. 3.1). The highest and lowest zones of Transect 1 and 2 were investigated twice at mid-July and end-August for the comparison of seasonal differences.

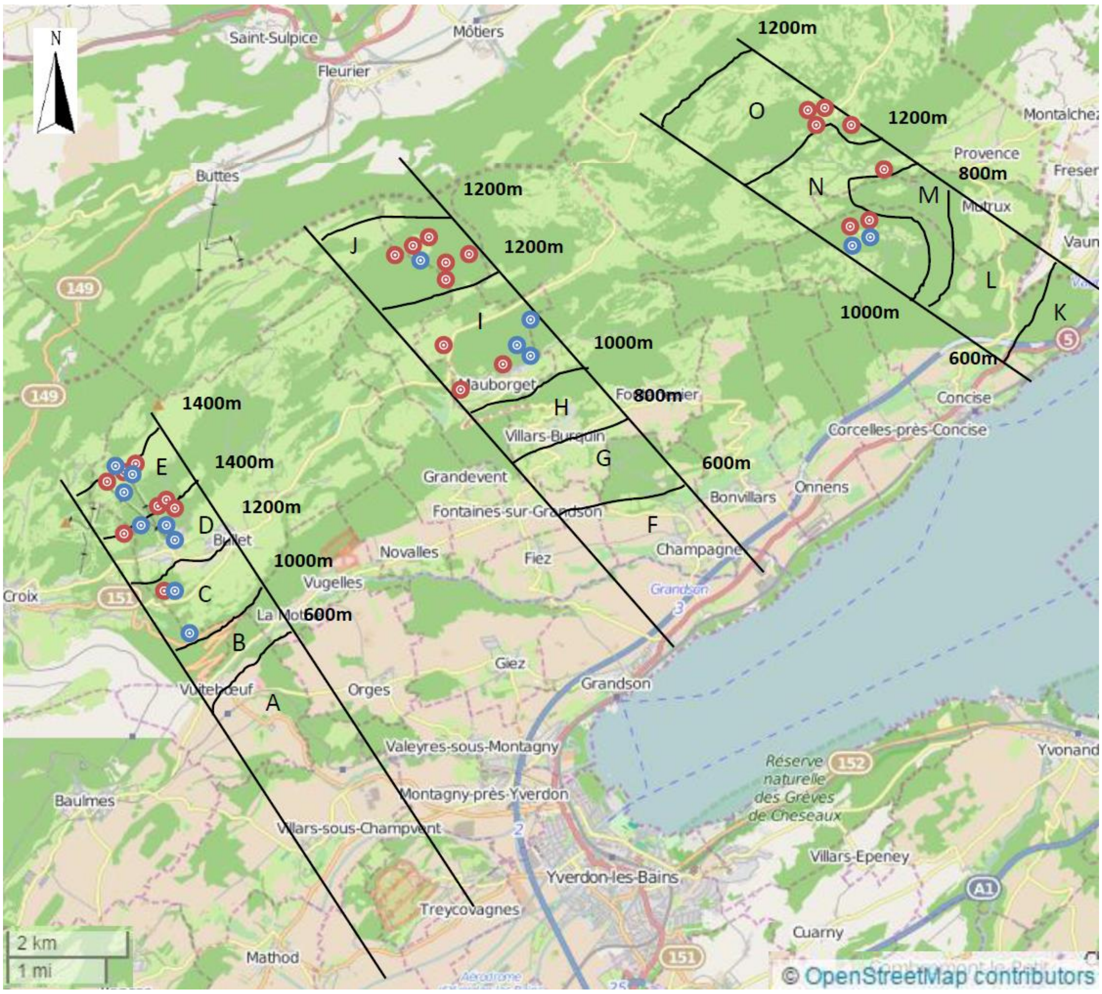

**Figure 3.1.** Three 2-km-wide transects with altitude ranges from 400 m to 1400 m at the Swiss Jura Mountains near Yverdon-les-Bains, Vaud, Switzerland. Letters A to O show the zones divided by contour line every 200 metres. Records of *Formica lugubris* are shown as red dots, and *F. paralugubris* as blue dots (© OpenStreetMap contributors).

## Methods

Two of the methods used for estimating nest size were the After-Disturbing method (a mark-release-recapture method to estimate worker population) and the Mound-Volume method (relative mound volume is calculated by multiplying two diameters and the height of the mound) which are both tested and proved to be accurate and feasible methods (Chen & Robinson, 2013). Total biomass of the nest was calculated by multiplying the average dry body mass and the worker population. For each zone, it took one day for surveying nests and marking ants, and one day for the recapturing process. I surveyed each section mainly along roads, paths and trails aiming to cover the whole section. Apart from private and inaccessible areas, the survey for each section focused on woodlands, deep forests and the edges between woodlands and pastures. In the surveying and marking day (the first day of mark-release-recapture method), I searched for nests without trails linking them to other nests in order to target monodomous nests of *F. lugubris*. Except for those were clearly polydomous, monodomous colonies of all sizes that I found were recorded. Once a nest was found, two basal diameters and the height of the nest mound were measured to calculate the mound volume. Survey date and time, nest location, the aspect of the nesting slope and air temperature were recorded. For the aspect, the south was defined as zero degree, western angle as positive degree and eastern angle as negative. Pictures of the nest and the surrounding environment were taken. Canopy cover of each nest was measured by photos, which were taken skyward above each nest (about 15 cm from the mound top) using a digital camera with a 28-mm focal length (S90, Canon, Japan), with the software ImageJ (version 1.48). Marking work of the After-Disturbing method was done within 15-20 min.

Recapturing work was usually done after two days. However, it was carried out after three days for four nests due to rain limiting ant activity. Temperatures of air and the inner nest were measured when recapturing work was in progress. A probe sensor of the thermometer was attached on a thin stick and poked inside the nest mound to measure the core temperature of the nest (approximately from 20 cm to 40 cm under the mound top) (Cherix *et al.*, 2006). After recapturing work of the After-Disturbing method was done, 50 individuals were collected after disturbing and preserved in 70% alcohol solution for identifying species and assessing body size and colony biomass. *Formica lugubris* and *F. paralugubris* were distinguished by the difference in the ratio of two measurements (Seifert, 1996): the length of the longest hair on lateral metapleuron and ventrolateral propodeum, and the width of the scale (petiole). Average head width (widest point) is a commonly used measurement of worker body size (Heinze *et al.*, 2003; Clémencet & Doums, 2007). Specimens were also oven-dried at 60-70$^{o}$C for 24 h to obtain average dry body mass (Tschinkel, 1993; Kaspari & Weiser, 1999).

### Statistical Analyses

Average dry body mass of each nest was assessed from the dry mass of 50 workers. Three indices were used to represent nest size: worker population (from After-Disturbing method), mound volume and total biomass. These indices of nest size were transformed by $\log_{10}$ to normalise the distribution. Pearson correlation coefficient was used for the relationship between worker population and mound volume. I used Generalised Linear Models (GLM) to test whether body size (head width), worker population, mound volume and total biomass changed along altitude, and whether there were other factors related to these indices of nest size. Nested two-way ANOVA were used to test whether there were seasonal differences in nest size.

Analyses were conducted with JMP statistics package (version 6.0.0; SAS institute, Cary, NC, USA) and R statistics package (version 3.1.0, R Development Core Team). To balance sample sizes for power analyses of altitudinal effect, I separated nests of *F. lugubris* into higher and lower groups by their altitude (above 1300 metres and below 1300 metres), and also separated nests of *F. paralugubris* into two groups by 1300 metres. In order to estimate required sample size for future studies, power analyses were conducted after t-tests for comparison of nest size and mound volume, and performed with G*Power 3.1.9 (Faul *et al.*, 2007).

## Results

I recorded 26 nests of *F. lugubris* and 15 of *F. paralugubris* (red and blue dots in Fig. 3.1). Worker population (from After-Disturbing method) and relative mound volume are positively correlated in *F. lugubris* and/or *F. paralugubris* (Fig. 3.2; *F. lugubris*: $r = 0.85$, $P < 0.001$, n = 26; *F. paralugubris*: $r = 0.89$, $P < 0.001$, n = 15; combining two species: $r = 0.88$, $P < 0.001$, n = 41).

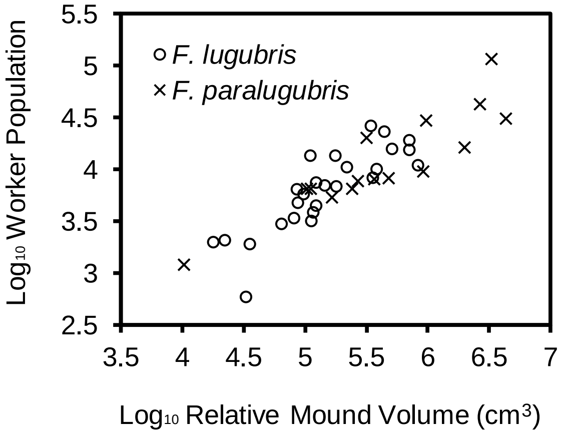

**Figure 3.2.** Positive correlation between worker population (from the After-Disturbing method) and relative mound volume (from the Mound-Volume method).

There was no significant relationship between altitude and any of the three indices of nest size in this study (Table 3.1, Fig. 3.3). Larger mounds tended to be found in shady areas rather than in open areas (Table 3.1). Nests located on slopes with a more western aspect were larger in worker population and total biomass than those on slopes with a more eastern aspect (Table 3.1). The interaction between canopy cover and the aspect of nesting slope had an effect on worker population, and had a borderline significant effect on total biomass (Table 3.1, Fig. 3.4): the difference in nest sizes of eastern-facing and western-facing slopes was greater when canopy cover was lower. *Formica paralugubris* nests were larger than *F. lugubris* in worker population and mound volume, and both species differed in total biomass at a borderline significant level.

**Table 3.1.** The effects of altitude, canopy cover and the aspect of nesting slope on worker population from After-Disturbing method (a), mound volume (b), and total biomass (c) of *Formica lugubris* and *F. paralugubris*. Generalised linear model (GLM) was used.

| (a) | Best model | | Best model with Altitude | |
|---|---|---|---|---|
| Parameter | Estimate | *t* value | Estimate | *t* value |
| Intercept | 3.75e+00 | 18.26*** | 3.21e+00 | 4.11*** |
| Altitude | | | 4.03e-04 | 0.73 |
| Species | 2.92e-01 | 2.25* | 2.86e-01 | 2.19* |
| Canopy Cover (C.C.) | 1.41e-03 | 0.46 | 1.98e-03 | 0.63 |
| Aspect (As.) | 7.57e-03 | 2.40* | 7.73e-03 | 2.43* |
| C.C.× As. | -1.02e-04 | -2.12* | -1.02e-04 | -2.12* |
| d.f. (residual/null) | 36/40 | | 35/40 | |
| AIC | 46.66 | | 48.04 | |

| (b) | Best model | | Best model with Altitude | |
|---|---|---|---|---|
| Parameter | Estimate | *t* value | Estimate | *t* value |
| Intercept | 3.62e+00 | 14.77*** | 3.15e+00 | 3.02** |
| Altitude | | | 3.42e-04 | 0.46 |
| Species | 4.50e-01 | 14.77* | 4.43e-01 | 2.53* |
| Canopy Cover | 7.92e-03 | 2.16* | 8.43e-03 | 2.18* |
| d.f. (residual/null) | 38/40 | | 37/40 | |
| AIC | 69.20 | | 70.97 | |

| (c) | Best model | | Best model with Altitude | |
|---|---|---|---|---|
| Parameter | Estimate | *t* value | Estimate | *t* value |
| Intercept | 1.04e+00 | 5.14*** | 2.09e-01 | 0.28 |
| Altitude | | | 6.21e-04 | 1.14 |
| Species | 2.60e-01 | 2.02# | 2.51e-01 | 1.96# |
| Canopy Cover (C.C.) | 2.46e-03 | 0.81 | 3.30e-03 | 1.06 |
| Aspect (As.) | 7.87e-03 | 2.52* | 8.13e-03 | 2.61* |
| C.C.×As. | -9.49e-05 | -1.97# | -9.58e-05 | -2.00# |
| d.f. (residual/null) | 35/39 | | 34/39 | |
| AIC | 44.36 | | 44.86 | |

C.C.×As.: Interaction of Canopy Cover and Aspect; # $P < 0.07$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

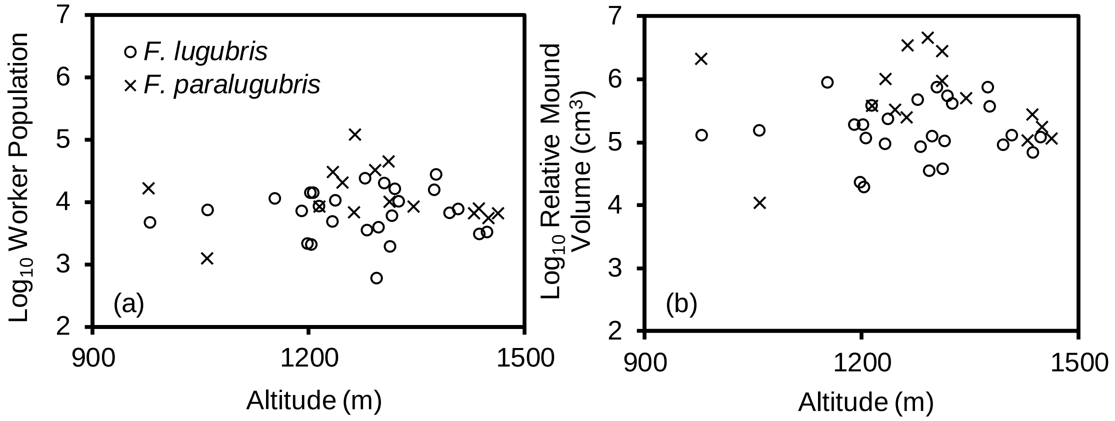

**Figure 3.3.** The relationships between altitude and two indices of nest size for *Formica lugubris* and *F. paralugubris*: worker population from After-Disturbing method (a) and relative mound volume (b). Statistical results see Table 3.1 (a) and (b).

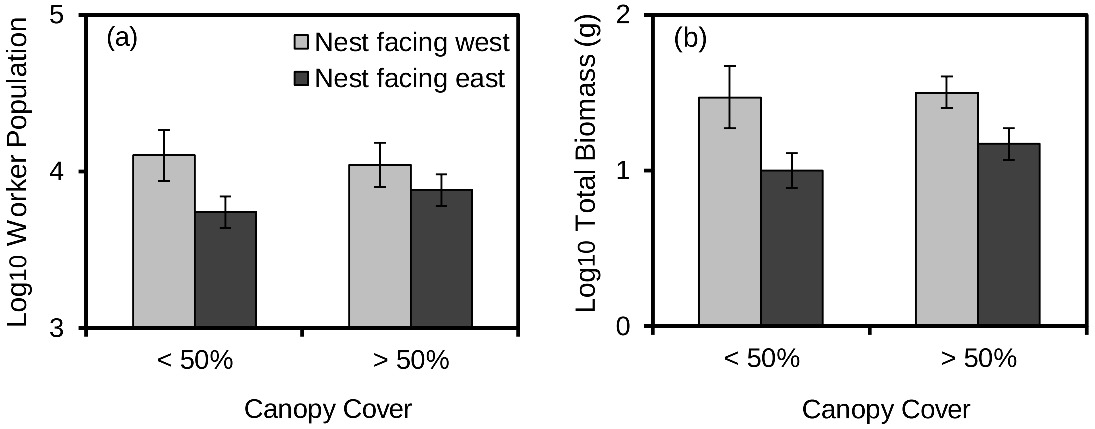

**Figure 3.4.** Interaction effect between canopy cover and the aspect of nesting slope on worker population from After-Disturbing method (a) and on total biomass (b) of *Formica lugubris* and *F. paralugubris*. Error bar: 1SE. Statistical results see Table 3.1 (a) and (c).

There was a borderline significant effect of the interaction between altitude and the aspect of nesting slope on body size (head width) (Table 3.2, Fig. 3.5): nests on slopes with a more eastern aspect tended to have increasing worker body size with increasing altitude, whereas worker body sizes of nests on slopes facing in a western direction tended to decrease with increasing altitude.

**Table 3.2.** The relationship between altitude, the aspect of nesting slope and body size (head width) of *Formica lugubris* and *F. paralugubris*.

| | Best model | |
|---|---|---|
| Parameter | Estimate | *t* value |
| Intercept | 7.50e+01 | 5.38*** |
| Altitude (Al.) | -7.53e-03 | -0.67 |
| Aspect (As.) | 6.39e-01 | 1.96# |
| Al.×As. | -4.86e-04 | -1.86# |
| d.f. (residual/null) | 37/40 | |
| AIC | 267.94 | |

Al.×As.: Interaction of Altitude and Aspect; # $P < 0.07$; *** $P < 0.001$

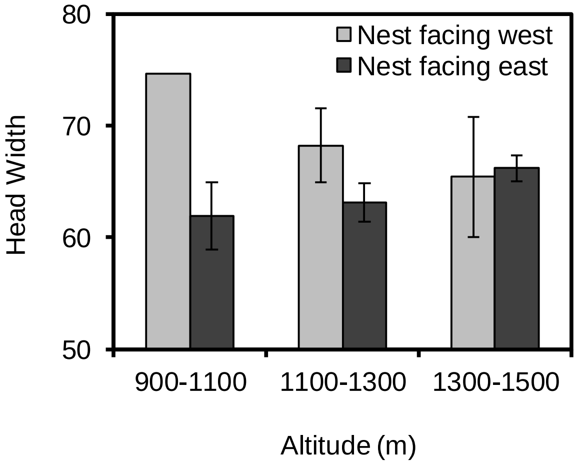

**Figure 3.5.** The interaction effect between altitude and the aspect of nesting slope on body size (head width) of *Formica lugubris* and *F. paralugubris*. Error bar: 1SE.

For the power analyses on the results of altitudinal effect, for *F. lugubris*, both worker population and mound volume were not significantly different between nests in higher (> 1300 m, n = 11) and lower altitude (< 1300 m, n = 15), however, the power of these analyses are low (worker population: $t$ = -0.96, $P$ = 0.35, two-tailed power = 0.15; mound volume: $t$ = -1.01, $P$ = 0.33, two-tailed power = 0.16). Using these results to predict what sample size would be required for a more reliable analysis, I found that 87 nests in each altitudinal range category are required to obtain a power of 0.70 for the analysis of difference in worker population, or 79 nests in each category for the analysis of mound volume. However, using one-tail t-test is justifiable because of the directional hypothesis of this study: colony size increases with increasing altitude. For one-tail t-tests, only 67 (for worker population) or 60 (for mound volume) nests of *F. lugubris* are required instead. In *F. paralugubris*, both worker population and mound volume were also not significantly different between nests in higher (> 1300 m, n = 7) and lower altitude (< 1300 m, n = 8) (worker population: $t$ = 0.85, $P$ = 0.41, two-tailed power = 0.12; mound volume: $t$ = -0.56, $P$ = 0.59, two-tailed power = 0.08). Sixty-eight nests are required in each altitudinal range category to obtain a power of 0.70 for the analysis of worker population or 155 nests for mound volume. Again, for one-tail t-tests, only 52 (for worker population) or 118 (for mound volume) nests of *F. paralugubris* are needed instead.

In terms of seasonal effects, worker populations of these two species in late-August were borderline larger than that in mid-July (Table 3.3). Mound volume of these two species was significantly larger in late August. The two species showed no difference in seasonal effect.

**Table 3.3.** Seasonal effect on worker population from After-Disturbing method and on mound volume of *Formica lugubris* and *F. paralugubris*. Nest identity is included as a random effect.

| | Worker Population | | | Mound Volume | | |
|---|---|---|---|---|---|---|
| Source | *F* ratio | d.f. | *P* value | *F* ratio | d.f. | *P* value |
| Nest[Species]&Random | 4.86 | 9 | < .05 | 17.73 | 12 | < .001 |
| Species (Sp.) | 0.11 | 1 | 0.74 | 0.12 | 1 | 0.73 |
| Seasonal Effect (Se.) | 4.41 | 1 | 0.07 | 20.23 | 1 | < .001 |
| Sp.×Se. | 0.04 | 1 | 0.84 | 0.69 | 1 | 0.42 |
| residual | | 9 | | | 12 | |
| $r^2$ adjust | | 0.65 | | | 0.89 | |

Sp.×Se.: Interaction of Species and Seasonal Effect

## Discussion

The results did not show a relationship between altitude and three nest size indices of *F. lugubris* and *F. paralugubris*. It is possible that the results really reflect a lack of relationship, however, I could not be confident in concluding that no relationship exists because of the following limitations in this study. The first limitation was the small range of altitude I obtained in the surveys. Although the altitude of the survey area ranged from 400 to 1500 metres, nests of *F. lugubris* and *F. paralugubris* were only found in a range between approximately 1000 and 1450 metres in altitude. This range may have been insufficient to show any difference of nest size along the geographic gradient. Therefore, I plan to perform the future study in the Swiss Alps for a larger altitudinal range. The lowest altitude nests were recorded at 1000 metres in this study and at 1100 metres in the Italian Alps (Bernasconi *et al.*, 2006). Previous records showed that these two species could be found at 1800 metres in the Swiss Alps (D. Cherix, personal communication) and 2100 metres in the Italian Alps (Bernasconi *et al.*, 2006). Because the highest altitude in the proposed site is 2200 metres, the expected altitudinal range of the study in the Swiss Alps should be at least 800 metres, i.e. almost twice the range achieved in the current study.

The other limitation was that the sample size of this study. I recorded only 26 nests (colonies) of *F. lugubris* and 15 of *F. paralugubris*. The analyses comparing the nest sizes in high and low altitude only had the power of 0.13-0.25 (one-tailed t-tests). According to the power analyses, I require a sample size of 60 to 67 nests in both low and high altitude areas (a total of 120 to 134 nests) for *F. lugubris* to reach the power of 0.70 (one-tailed analyses). The feasibility of obtaining the requisite sample size can be increased by reducing the time consumed in measuring nest size. The After-Disturbing method is accurate but requires two days to obtain the nest size of each nest. The total biomass method needs not only worker population data but also body mass of workers. In contrast, the Mound Volume method takes less than three minutes for a given nest because only the diameters and the height of the mound are needed. I plan to use Mound Volume method to estimate nest size in future studies for obtaining a sufficient sample size in a limited time frame. Although the Mound-Volume method is not as accurate as the After-Disturbing method is (Chen & Robinson, 2013), the results of the Mound-Volume method do correlate strongly with the After-Disturbing method at this site (Fig. 3.2, Table 3.1). According to the power analyses, a required sample size of 120 *F. lugubris* nests and 236 *F. paralugubris* nests should be obtained with Mound-Volume method.

The study also showed that other environmental factors were related to nest size. The effect of canopy cover either acted alone or in combination with the effect of slope aspect on each index of nest size. Canopy cover strongly influences the thermal environments of the locations on a woodland floor (Rodriguez-Garcia *et al.*, 2011; van Gils & Vanderwoude, 2012). Thus, a study which specially focuses on the effect of canopy cover on wood ant nests will be performed (Chapter 4). Moreover, the aspect of nesting slope was also related to worker population and total biomass. I thus need an accurate and time-saving method to take into account the direction where sunshine comes from. Using canopy cover images from fisheye lens can be the solution for the measurements of both canopy cover and the aspect of nesting slope. Fisheye lens obtains a circular photo covering a 180-degree angle of view. Compared with the image from 28-mm-focal-length lens I used in this study, the image from fisheye lens can include not only a wide and complete range but also the unique shape of the canopy cover for each nest (Frazer *et al.*, 1999). Therefore using fisheye lens for the measurement of canopy cover will fit in with my needs of the assessments on both canopy cover and the aspect of nesting slope.

I attempt to apply Bergmann's rule from latitude to altitude because of the similarity on changes of thermal patterns along the gradients. The thermal environments at the forest floor layer are also influenced by canopy cover (Rodriguez-Garcia *et al.*, 2011; van Gils & Vanderwoude, 2012). In order to test whether temperature is the mediating factor working on nest size through altitude and canopy cover, I plan to check the effects of canopy cover and altitude on local temperature by collecting thermal data at the nest locations. Data loggers (iButton: DS1921G-F5; Maxim/Dallas Semiconductor, TX, US) will be used to continuously record local temperature for the analyses of daily mean, maximum and minimum temperatures.

Nests of *Formica paralugubris* seemed to be larger than those of *F. lugubris* in three indices of nest size. The difference of nest sizes between two species may result from the sampling method of this study. I targeted monodomous nests in order to find *F. lugubris* colonies (for a monodomous colony, a nest is a colony). Some nests of *F. paralugubris* were included due to the lack of trails between nests during the surveys.

Because I did not sample these *F. paralugubris* nests randomly, the difference of nest sizes in this study may not actually show the difference between these two species. In the future study of the relationship between altitude and colony size in the Swiss Alps (Chapter 5), I plan to target both *F. lugubris* and *F. paralugubris* because: 1) I am not able to identify them from each other morphologically in the field; 2) both species are polydomous in the Swiss Alps and cannot be distinguished by domy forms. Workers will be collected from each nest for confirming species identification.

In terms of body size, I did not find Bergmann's cline between altitude and body size (head width, Table 3.3). Again, just as I did not find the cline between colony size and altitude, the same limitations of low altitudinal range and low sample size may apply to altitude and body size. Heinze *et al.* (2003) reported that two high altitude populations of *Leptothorax acervorum* had larger worker body size than expected from their latitude, but he did not show altitude information for the populations. In this study, the nesting slopes facing different aspects may have different effects on body size (Fig. 3.5). I suppose that slopes with an eastern aspect receive solar radiation much earlier than those with a western aspect every day. Workers of the nests on slopes with an eastern aspect could start being active early and keep inner-nest temperatures over the active level for a long time. For the eastern-facing nests, increasing altitude may considerably influence the length of time with active temperatures and result in a larger body size. In contrast, the nests on slopes with a western aspect may always receive little sunshine regardless of the altitude they located at. Therefore, the thermal environments within the western-facing nests may be not strongly associated with altitude (Fig. 3.5). I therefore suggest that, compared to the results of colony size, body size is more sensitive than colony size to the small change on thermal environments linked with altitude (Table 3.1 and 3.2).

With regard to seasonal effects, nest size was detected to grow within two months in the active season. If the period of a survey is more than two months, the results of nest size could be influenced by seasonal effects. According to the required sample size calculated from power analyses, I should record more than 350 nests. Future study may proceed across more than two months for obtaining the requisite sample size. Therefore, sampling date may also be considered for the analyses of nest size.

To sum up, for future study of the relationship between altitude and colony size, I plan to conduct the investigation on both *F. lugubris* and *F. paralugubris* in the Swiss Alps for an altitude range of at least 800 metres (Chapter 5). Polydomous organisation of the colony will be considered for statistical analyses. Mound volume will be used to estimate nest size for the best balance of accuracy, effort, and time consumed. According to the power analyses, about 120 nests of *F. lugubris* and 236 nests of *F. paralugubris* will be needed. The effect of canopy cover not only will be the focus of a direct study investigating its impact on nest size (Chapter 4) and will also be taken into consideration when altitude is studied (Chapter 5). The measurement of canopy cover will be done with the photos taken by fisheye lens for complete information of the canopy. I will also record local temperatures by data loggers to check the links between thermal environments, altitude and canopy cover in order to understand whether temperature is the mediating factor working on nest size.

# Chapter 4 – The relationship between canopy cover and colony size of the wood ant *Formica lugubris* - implications for the thermal effects on a keystone ant species

## Abstract

Climate change may affect ecosystems and biodiversity through the impacts of rising temperature on species' body size. In terms of physiology and genetics, the colony is the unit of selection for ants so colony size can be considered the body size of a colony. For polydomous ant species, a colony is spread across several nests. This study aims to clarify how climate change may influence an ecologically significant ant species group by investigating thermal effects on wood ant colony size. The strong link between canopy cover and the local temperatures of wood ant's nesting location provides a feasible approach for our study. Our results showed that nests were larger in shadier areas where the thermal environment was colder and more stable compared to open areas. Colonies (sum of nests in a polydomous colony) also tended to be larger in shadier areas than in open areas. In addition to temperature, our results supported that food resource availability may be an additional factor mediating the relationship between canopy cover and nest size. The effects of canopy cover on total colony size may act at the nest level because of the positive relationship between total colony size and mean nest size, rather than at the colony level due to lack of link between canopy cover and number of nests per colony. Causal relationships between the environment and the life-history characteristics may suggest possible future impacts of climate change on these species.

# Introduction

Climate change is one of the most notable ecological and environmental issues. This phenomenon, which is of global concern, has altered species distribution and abundance, and consequently affected ecosystems and biodiversity (Parmesan, 1996; Hughes, 2000; Smith & Smith, 2003; Forero-Medina *et al.*, 2011). There are many predictions for climate change, including more frequent storms and hurricanes, and greater snowfall. Rising average and extreme temperatures are the main and general predictions (Smith & Smith, 2003). For plants and many ectotherms, temperature has a profound impact on many functions relating to an organism's size, such as metabolic rates and rates of gas exchange (Atkinson, 1994). Body size is probably the most significant life-history characteristic of an animal due to its influence on most physiological and morphological characters (Atkinson, 1994; Brown & Lomolino, 1998; Blanckenhorn & Demont, 2004). Therefore, climate change may affect animals through impact on body size mediated by rising temperature (Smith *et al.*, 1995; Hunt & Roy, 2006).

For social insects, the colony can be considered the biological analogue of the body of a solitary organism (Kaspari & Vargo, 1995; Tschinkel, 1998, 1999). Colony size of social insects has been represented by the total number of individuals or workers in a colony (Kaspari & Vargo, 1995; Tschinkel, 1998, 1999). Just as body size has a significant role for solitary organisms, colony size has been known to correlate with the lifestyle of a social insect colony, for example, competitive abilities, foraging behaviours and life span (O'Donnell & Jeanne, 1992; Pamilo *et al.*, 1992; Eckert *et al.*, 1994; Herbers & Choiniere, 1996; Palmer, 2004). Again, just as for body size, temperature is one of the exogenous factors which affects colony size in social insects (Porter & Tschinkel, 1993; Asano & Cassill, 2012). For these reasons, colony size could be a useful index to understand how climate change will influence social insects.

The red wood ants are a group of morphologically similar *Formica* species (Cotti, 1996; Bernasconi *et al.*, 2011), which are ecologically dominant and have impacts at multiple community levels including ants, other arthropods and vertebrates, across northern Eurasia (Sudd & Lodhi, 1981; Savolainen & Vepsäläinen, 1988; Haemig, 1992; Punttila *et al.*, 1994; Rolstad *et al.*, 2000; Punttila *et al.*, 2004; Kilpeläinen *et al.*, 2005). Red wood ants can affect the growth of trees both negatively, by herding sap-sucking aphids, and positively, by increasing predation or harassment of other herbivores (Rosengren & Sundström, 1991; Styrsky & Eubanks, 2007). They build nests with large aboveground mounds which function as habitats for myrmecophiles and influence the nutrient cycle of the forest (Laakso & Setälä, 1997, 1998; Domisch *et al.*, 2008; Jurgensen *et al.*, 2008; Robinson & Robinson, 2013). They are also ecological indicators for land-use changes in European broadleaf forest and taiga (Ellison, 2012). Red wood ants have significant impacts on forest ecosystems and most of them are considered “near threatened” by the International Union for Conservation of Nature (IUCN, 2014). Furthermore, because future climate change predictions also indicate more severe warming at higher latitudes (Walther *et al.*, 2002; ACIA, 2004), understanding how climate change may affect these temperate species is therefore important for future conservation actions.

Species distribution modelling and physiological experiments have been the prevailing research for the potential effects caused by climate change. Temperature experiments such as testing thermal tolerance can be an useful tool for modelling and predicting responses of ants to warming (Diamond *et al.*, 2013). For ants, some species-level studies have asserted the negative impacts on physiology or behaviours from climate change (Andrew *et al.*, 2013; Diamond *et al.*, 2013; Stuble *et al.*, 2013); others have revealed its promotive role on the expansion of species distribution, especially for invasive species (Roura-Pascual *et al.*, 2004; Morrison *et al.*, 2005; Chen, 2008; Diamond *et al.*, 2012; Bertelsmeier *et al.*, 2013a; Bertelsmeier *et al.*, 2013b).

Although species' responses to specific environmental factors such as temperature can be tested in laboratories, a laboratory approach may not be effective for capturing the effects caused by daily or annual dynamics of temperature. It would be more comprehensive if we can directly investigate these in the field, if conditions accurately representing the natural environment cannot be simulated. This could be achieved by a field transplant or a common garden experiment (Pelini *et al.*, 2012). However, as for many social insects, red wood ant nests are complex and long-lasting. Wood ants spend many years building large nest mounds in woodland, and one red wood ant colony may also settle in several spatially separated but socially connected nests, called polydomy (Debout *et al.*, 2007; Ellis & Robinson, 2014). It is not feasible to move the whole colony without damage and long-term effects on the colony's function and organisation.

Fortunately, it is known that the thermal environments of the locations on a woodland floor are strongly influenced by canopy cover (Rodriguez-Garcia *et al.*, 2011; van Gils & Vanderwoude, 2012). This provides a practicable approach to explore how colony size and nest size are related to a lasting but localised thermal environment, which a red wood ant colony may continually experience for years.

Moreover, in addition to temperature, higher canopy cover may imply more surrounding trees, which probably provide more aphids, the main food resource of red wood ants. Food resource availability may positively relate to wood ant nest size (Sorvari & Hakkarainen, 2005; Punttila & Kilpeläinen, 2009). Therefore, we might be able to detect the role of food resource availability in the relationship between canopy cover and nest size.

In this study, we investigated the relationship between canopy cover and both the total colony size (worker population of a polydomous colony) and nest size (worker population of a single nest) of a woodland specialist ant species in the field. There is a known negative relationship between canopy cover and temperature (Geiger *et al.*, 2009; Rodriguez-Garcia *et al.*, 2011; Huang *et al.*, 2014); we verified this at our site by collecting thermal data at the colony locations. Larger nests or colonies are expected to cope better with colder environments due to increased abilities to regulate inner nest temperature (Rosengren *et al.*, 1987; Punttila & Kilpeläinen, 2009). We would therefore expect to observe larger colony size and nest size in shady areas with a colder environment.

## Materials and Methods

### Species and Location

The study species was the red wood ant *Formica lugubris* (Hymenoptera: Formicidae). To focus on the relationship between canopy cover and colony size, and to minimise the effects from altitude and slope direction, we conducted our study in a part of the Longshaw Estate, Peak District (53°18'35"N, 01°36'25"W; access permission obtained with S. Ellis by the National Trust) in the UK. It is a flat area

(~1.1 $km^2$) with an altitudinal range of 270-350 m. *Formica lugubris* has both monodomous and polydomous social forms (Bernasconi *et al.*, 2005; Maeder *et al.*, 2005; Ellis & Robinson, 2014), and is polydomous in Great Britain (Sudd *et al.*, 1977; Ellis & Robinson, 2014). We defined a polydomous colony as a group of nests which are connected each other by trails. There are over 900 nests of polydomous *F. lugubris* in our sampling area, and the number of nests per colony ranging from 1 to 22 nests (Ellis *et al.*, 2014).

## Methods

The study was conducted in June 2013, when canopy cover had reached a relatively stable level. To choose colonies to include in our sample, we divided the experimental site into a grid of 44 squares with a side length each of 140 metres. We defined the intersections of the gridlines as our sampling points. We located the nest nearest to each sampling point and the colony to which this nest belonged was chosen for inclusion. Because the longest distance between two nests of the same polydomous colony was 52 metres (2.5 metres on average, more than 90% trails below 8 metres, S. Ellis, preliminary survey), by this method, we minimised the chance of choosing a colony that included several nests within different sampling points. We defined a sampling point as having no colony present if we could not find any nest within a radius of 70 metres from the intersection. This sampling method was able to include a range of canopy cover (from an isolated tree to dense cover).

We mapped the chosen colonies, recording: the number of and size of nests; spatial distribution pattern of nests; the trails between nests; foraging trails between nests and trees. In addition, number of inter-nest trails per nest, trail length and number of forage trees used by each nest were recorded. In our study, we defined a distinct trail from a nest to a tree as a foraging trail (see (Ellis *et al.*, 2014)). However, it does not mean that the nests without any obvious foraging trails were not foraging at all; they might be involved in other foraging activities. A Mound-Volume method was used to estimate nest size; three dimensions of nest mound were multiplied to represent the total number of individuals of mound-building wood ants (Eeva *et al.*, 2004; Punttila & Kilpeläinen, 2009; Chen & Robinson, 2013). This method has been tested and shown to provide a reliable estimate of nest worker population in this species (Chen & Robinson, 2013). A photo was taken skyward above each nest using 180-degree hemispherical lens (FC-E8 fisheye lens with Coolpix 5000, Nikon Corporation, Tokyo, Japan) which produces circular images that record the size, shape, and location of gaps of the canopy. Canopy cover (percentage) was estimated from the circular photo using the software Gap Light Analyzer 2.0 (Frazer *et al.*, 1999).

For the background thermal environment, we derived annual solar radiation data from digital elevation model data at 10-metre resolution (© Crown Copyright 2014. An Ordnance Survey/EDINA supplied service.). The calculation was done using the Area Solar Radiation tool in the Spatial Analyst toolbox of ArcMap 10.1 and specifying the latitude, elevation and slope direction of our sampling points. The calculation sampled every day throughout 2013, using a 30-minute interval. All other settings were set to default. Besides the annual solar radiation as background data, we also wanted to obtain information about the small-scale thermal environment of the nest. For this reason, a temperature-recording device was placed on the ground next to the north side (to reduce the chance of direct sunshine exposure) of the nest which was discovered first in every colony. The devices consisted of a polyethylene terephthalate (PET) tube (diameter = 10 cm, length = 20 cm) wrapped in aluminium foil to reduce the effect of direct solar radiation (Suggitt *et al.*, 2011). A thermal datalogger (iButton: DS1921G-F5; Maxim/Dallas Semiconductor, TX, US) was placed in each device to record hourly environmental temperatures from 31st May for 16 days.

## Statistical Analyses

Total colony size was calculated as the sum of nest sizes to represent the total number of individuals in a colony. Size data (nest size and total colony size) were transformed by $\log_{10}$ to normalize the distributions. We used “lme” function from the “nlme” package for R (version 3.0.1, R Development Core Team) to fit linear mixed-effect models for: 1) the effects of annual solar radiation and canopy cover on nest size; 2) the relationships of the number of nests per colony to canopy cover and nest size; 3) whether the presence or absence of foraging trail was related to canopy cover and nest size; and 4) the relationships of foraging trail length to canopy cover and nest size. For linear mixed-effect models, the best model was selected according to AIC and the significance of factors. Colony identity was included as a random effect in the models.

Linear regression models were used for: 1) the effects of annual solar radiation and canopy cover on total colony size; 2) the relationship between the size of the largest nest of each colony and canopy cover, and between the size of the smallest nest of each colony and canopy cover; 3) the relationship between total colony size and mean nest size per colony; 4) the relationship of annual solar radiation, number of nests per colony and canopy cover to six local temperature parameters- the mean and the standard deviation of hourly temperature ($\mathrm{Temp}_{\mathrm{Mean}}$ and $\mathrm{Temp}_{\mathrm{SD}}$), the mean and the standard deviation of daily maximum and minimum temperature ($\mathrm{Max}_{\mathrm{Mean}}$, $\mathrm{Max}_{\mathrm{SD}}$, $\mathrm{min}_{\mathrm{Mean}}$, and $\mathrm{min}_{\mathrm{SD}}$). For linear regressions, F test was used to select the best model. Pearson's correlation was used for total colony size and six local temperature parameters. If a temperature parameter was correlated to both total colony size and canopy cover, partial correlation was used to measure the degree of association between total colony size and canopy cover, with the effects of this temperature parameter removed.

To analyse the variation in nest size at different levels of canopy cover, nests were separated into three groups based on the canopy cover of their location to balance the sample size of each group: nests with canopy cover lower than 51.2% (n = 67), between 51.2% and 67.5% (n = 67), and higher than 67.5% (n = 67). To analyse the differences of total colony size between colonies, we also separated colonies into three groups based on their number of nests to balance the sample size: colonies with one to three nests (Close-to-Monodomous Group, n = 12), colonies with four to seven nests (Intermediate-Polydomous Group, n = 12), and colonies with more than seven nests (Polydomous Group, n = 10). Levene's tests were used to compare the variances between groups. Kruskal-Wallis' test was used to compare total colony size of each group. Linear regression models, Levene's test and Kruskal-Wallis' test were conducted with the JMP statistics package (version 6.0.0; SAS institute, Cary, NC, USA).

## Results

Thirty-four colonies, with a total of 201 nests, were sampled and recorded for this study. There was no colony at 10 sampling points. We found that nest size increased significantly with increasing canopy cover (linear mixed-effect model, solid line in Fig. 4.1, fixed effect: $t = 2.19$, $P < 0.05$, $n = 201$, reduced model AIC = 464.23). The full model contained two factors: canopy cover and annual solar radiation, the latter factor had no significant effect on nest size (t = -0.69, $P = 0.50$, n = 201, full model AIC = 489.40). There was no significant relationship between the size of the largest nest of each colony and canopy cover (linear regression: $F = 2.64$, d.f. = 33, $P = 0.11$, see Fig. 4.1), or between the size of the smallest nest of each colony and canopy cover (linear regression: $F = 3.14$, d.f. = 33, $P = 0.09$, see Fig. 4.1). The variances of nest size did not significantly differ between three groups with different canopy cover (Levene's test, $F = 0.72$, $P = 0.49$, n = 67 for each group). Total colony size, which was the sum of the size of all nests in that colony, borderline significantly increased with increasing mean canopy cover (linear regression: $F = 3.67$, d.f. = 33, $P = 0.06$, reduced model $r^2 = 0.10$, Fig. 4.2). Again, the factor annual solar radiation did not have a significant effect on colony size (t = -1.18, $P = 0.25$), and did not significantly improve the model (full model $r^2 = 0.19$, F test, $F = 1.17$, $P = 0.25$). Canopy cover at our 201 sampled nests ranged from 24% to 86%, with a mean of 59%.

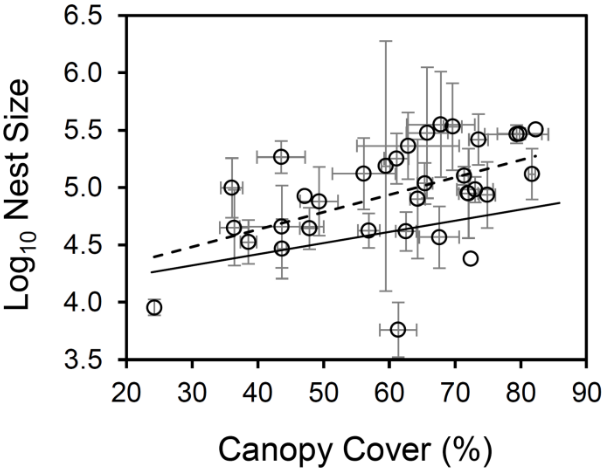

**Figure 4.1.** The relationship between mean nest size and mean canopy cover. Circle dots: the log10 mean nest size and mean canopy cover of 34 colonies; grey error bar: 1SE, four points without error bars are colonies containing only one nest; dashed line: $y = 0.0149\,x + 4.0423$, $F$ ratio = 11.10, $P < 0.001$, $r^2 = 0.26$, model fitted by linear regression for the relationship between mean nest size

and mean canopy cover; solid line: $y = 0.0097\ x + 4.0282$, from the fixed effects of the linear mixed-effect model, which includes colony identity as a random effect. Full analyses are showed in results.

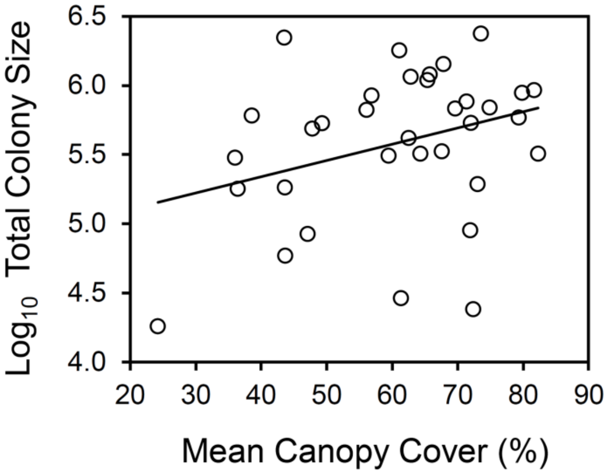

**Figure 4.2.** The trend between total colony size and mean canopy cover of 34 colonies. Linear regression, solid line: $y = 0.0117\ x + 4.8727$, $F$ ratio = 3.67, $P = 0.06$, $r^2 = 0.10$.

One temperature-recording device was lost. According to the records of the 33 nests from which dataloggers were retrieved, both Temp$_{\text{Mean}}$ and the Temp$_{\text{SD}}$ of local environmental temperatures were lower with increasing canopy cover (linear regressions: Fig. 4.3). Max$_{\text{Mean}}$ were also lower in shadier areas, whereas there was no significant relationship between the Max$_{\text{SD}}$ and canopy cover. Min$_{\text{Mean}}$ increased with rising canopy cover, whereas min$_{\text{SD}}$ decreased (Fig. 4.3). The relationships between the local temperature parameters and total colony size were similar to the relationships between the local temperature parameters and canopy cover: there were negative correlations of total colony size with Temp$_{\text{Mean}}$, Temp$_{\text{SD}}$, Max$_{\text{Mean}}$ and min$_{\text{SD}}$, whereas min$_{\text{Mean}}$ was borderline significantly positively correlated with total colony size. There was no significant correlation between total colony size and Max$_{\text{SD}}$ (Table 4.1). For three-way correlation between total colony size, canopy cover and the local temperature parameters, using partial correlation to remove the effects of the local temperature parameters eliminated the positive trend between total colony size and canopy cover (Table 4.1). Annual solar radiation levels showed no significant relationship with the six local temperature parameters (Annual solar radiation: 873978.25 ± 26008.40, whr/m$^2$, Mean ± SD, linear regressions: $F$ = 0.02-0.94, d.f. = 32, $P$ = 0.33-0.89).

**Table 4.1.** Correlations and partial correlations between canopy cover, total colony size and six local temperature parameters (the mean and the standard deviation of hourly temperature , $\text{Temp}_{\text{Mean}}$ and $\text{Temp}_{\text{SD}}$; the mean and the standard deviation of daily maximum and minimum temperature, $\text{Max}_{\text{Mean}}$, $\text{Max}_{\text{SD}}$, $\text{min}_{\text{Mean}}$, and $\text{min}_{\text{SD}}$).

| | Canopy Cover | Temperature Parameters | | Partial Correlation# |
|---|---|---|---|---|
| Total Colony Size | 0.31$^{!}$ | $\text{Temp}_{\text{Mean}}$ | -0.44* | 0.10 |
| | | $\text{Temp}_{\text{SD}}$ | -0.40* | 0.10 |
| | | $\text{Max}_{\text{Mean}}$ | -0.39* | 0.16 |
| | | $\text{Max}_{\text{SD}}$ | -0.10 | - |
| | | $\text{min}_{\text{Mean}}$ | 0.31$^{!}$ | 0.14 |
| | | $\text{min}_{\text{SD}}$ | -0.45** | -0.13 |

$^{!}P = 0.08$, *$P < 0.05$, **$P < 0.01$, #Partial correlation between total colony size and canopy cover with the effects of local temperature parameters removed, n = 33.

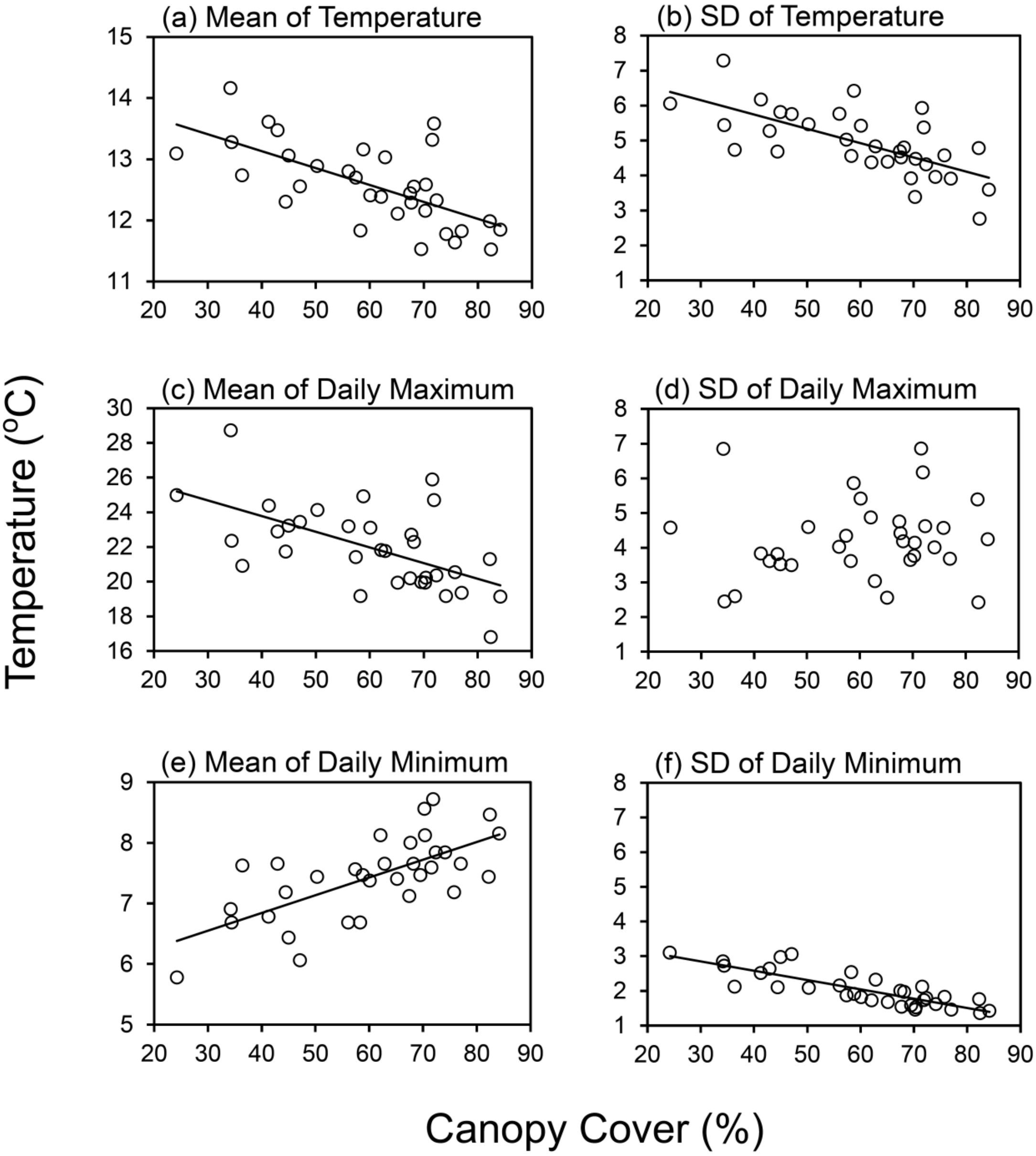

**Figure 4.3.** The relationships between canopy cover and six local temperature parameters for 33 colonies. Solid line of each graph shows the significant model fitted by linear regression. (a) the mean of temperature: $y = -0.03\ x + 14.24$, $P < 0.001$, $r^2 = 0.43$. (b) the standard deviation (SD) of temperature: $y = -0.04\ x + 7.38$, $P < 0.001$, $r^2 = 0.46$. (c) the mean of daily maximum temperature: $y = -0.09\ x + 27.39$, $P < 0.001$, $r^2 = 0.34$. (d) the SD of daily maximum temperature: not significant. (e) the mean of daily minimum temperature: $y = 0.03\ x + 5.67$, $P < 0.001$, $r^2 = 0.46$. (f) the SD of daily minimum temperature: $y = -0.03\ x + 3.66$, $P < 0.001$, $r^2 = 0.69$.

The sizes of nests with at least one foraging trail was greater than those of nests without any foraging trail (linear mixed-effect model, fixed effect: t = 4.70, $P < 0.001$, n = 201, model AIC = 441.93, Fig. 4.4). Nests with foraging trail/s were also located in areas with higher canopy cover than those without any foraging trail (with foraging trail/s: 61.83% ± 3.73, without foraging trail: 56.71% ± 2.62, Mean ± SE, linear mixed-effect model, fixed effect: t = 4.57, $P < 0.001$, n = 201, model AIC = 1443.27). The minimum length of foraging trails decreased with an increase of canopy cover (minimum length of foraging trails: 4.52m ± 0.33, Mean ± SE, linear mixed-effect model, fixed effect: t = -4.44, $P < 0.001$, n = 135, model AIC = 979.40). There was no relationship between minimum foraging trail length and nest size (linear mixed-effect model, fixed effect: t = -0.66, $P < 0.51$, n = 135, model AIC = 309.46).

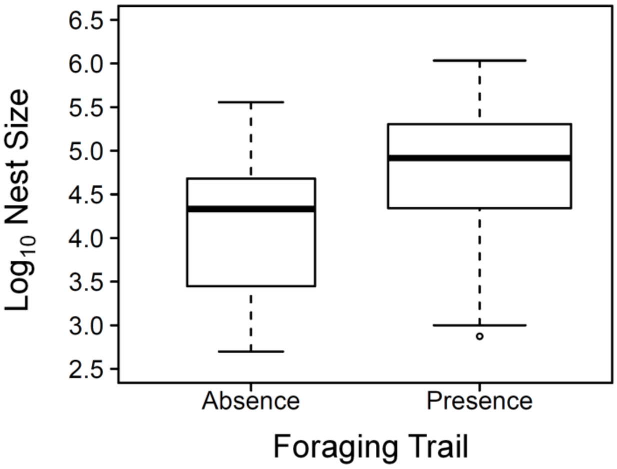

**Figure 4.4.** The relationship between nest size and the presence or absence of foraging trail. Boxplots show the range, quartiles, medium and outliers of the data. Boxplot width is proportional to the square root of sample size. This figure does not take colony identity in account, but the full analysis does (linear mix-effect model).

Colonies included in this study ranged from a single nest (monodomous) to as many as 20 nests connected as a single polydomous colony. Total colony size of a polydomous colony could be larger through one or both of the following ways: have bigger individual nests, or have more nests per colony. Our results showed that colonies with greater total colony size had greater mean nest size (mean nest size value as Fig. 4.1 shows, $F = 40.41$, d.f. = 33, $P < 0.001$, $r^2 = 0.56$). On the other hand, total colony size also increased when a colony had more nests; there was a significant increase in total colony size from Close-to-Monodomous Group (with one to three nests, n = 12) to Intermediate-Polydomous Group (with four to seven nests, n = 12) and Polydomous Group (with more than seven nests, n = 10) (Kruskal-Wallis' test, $\chi^2 = 10.15$, $P < 0.01$, Fig. 4.5). The groups did not significantly differ in the variances of total colony size from each other (Levene's test, $F = 1.88$, $P = 0.16$). As for the two factors which are related to total colony size, we found a borderline significant negative relationship between nest size and the number of nests per colony (linear mix-effect model, fixed effect: t = -2.03, $P = 0.051$, n = 201, model AIC = 462.33, Fig. 4.6). There was no significant relationship between canopy cover and number of nests per colony (linear mix-effect model, fixed effect: t = -1.00, $P = 0.33$, n = 201, model AIC = 1463.63). Number of nests per colony showed no significant relationship with the six local temperature parameters (linear regressions, $F = 0.00\text{-}0.38$, d.f. = 32, $P = 0.54\text{-}0.95$). Annual solar radiation showed no significant relationship with the six local temperature parameters (linear regressions, $F = 0.02\text{-}0.95$, d.f. = 32, $P = 0.34\text{-}0.88$). We made a flow chart showing the relationship between canopy cover, nest size, colony size and other factors in the present study (Fig. 4.7).

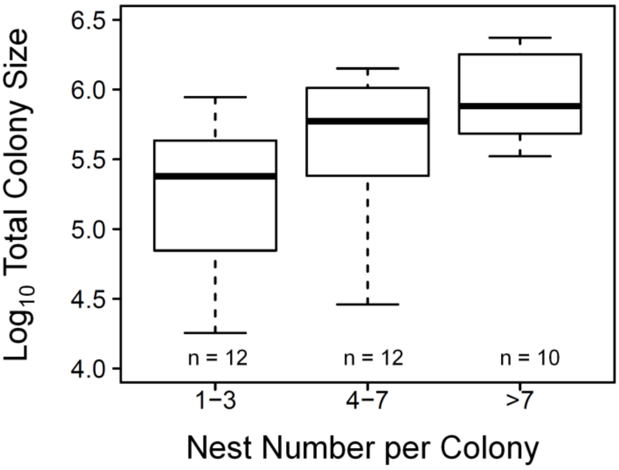

**Figure 4.5.** The relationship between number of nests and total colony size. Kruskal-Wallis' test, $\chi^2 = 10.15$, $P < 0.01$.

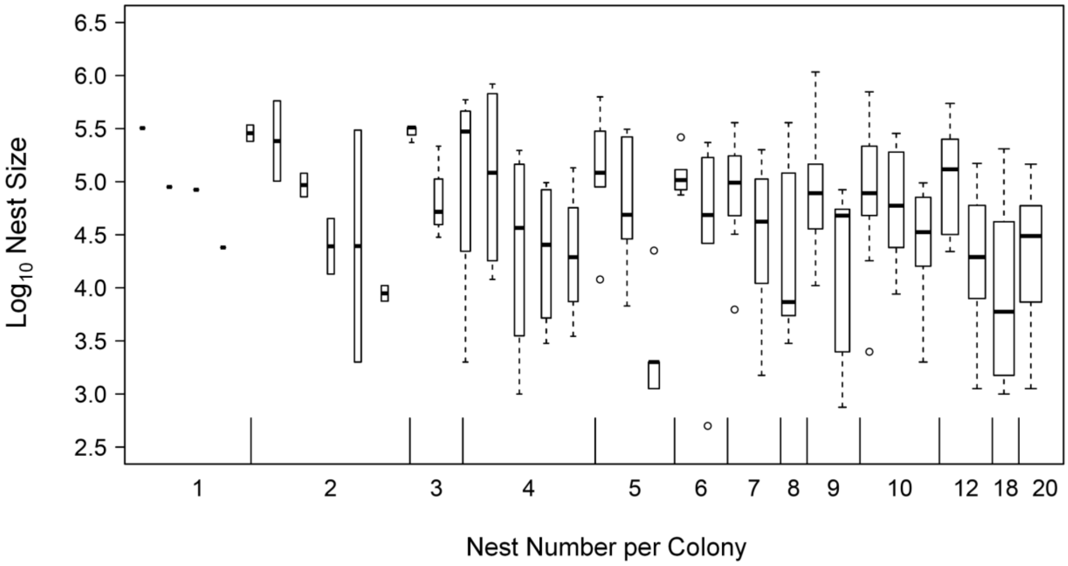

**Figure 4.6.** The relationship between number of nests per colony and nest size. Nest size tends to decrease as number of nests per colony increases (Linear mixed effect model, fixed effect: t = -2.03, $P = 0.051$, model AIC = 462.33). Boxplot width is proportional to the square root of number of nests.

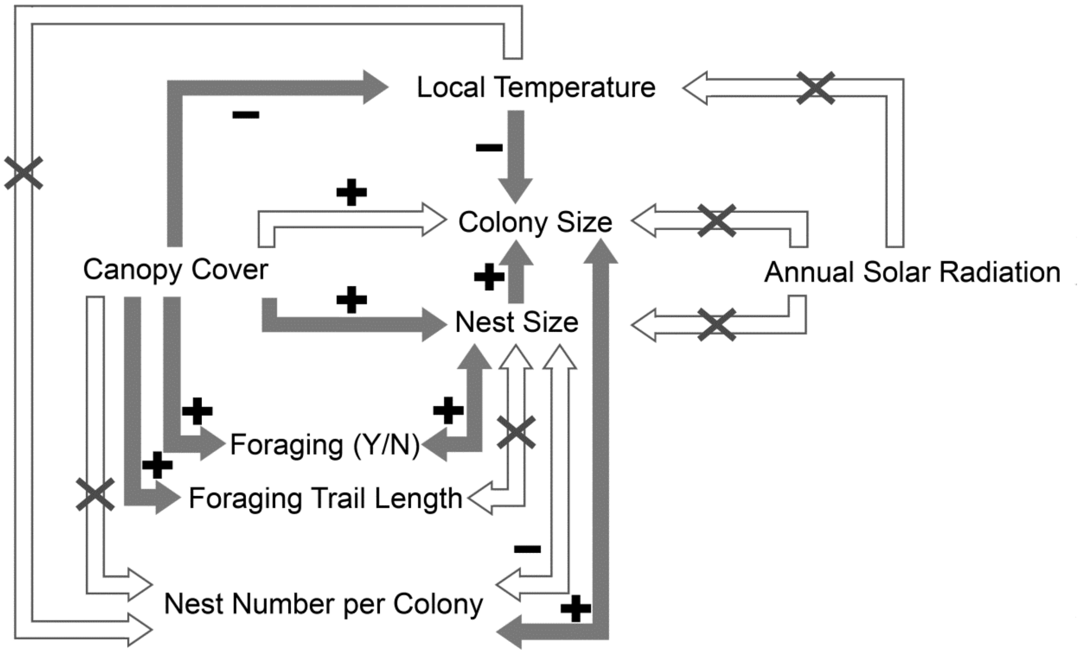

**Figure 4.7.** The relationships of colony size and nest size to possible related factors hollow arrow with a cross indicate the significant, the borderline significant and the non-significant relationships respectively. Plus and minus signs indicate the relationships as positive and negative respectively.in our study. Arrows illustrate the possible direction of causality. Solid arrow, hollow arrow and

## Discussion

Our results clearly showed that red wood ant (*F. lugubris*) nest size increased with increasing canopy cover: nests in shady areas were larger than those in open areas (Fig. 4.1). Temperature and food resources are two important factors which would be predicted to affect nest size and are related to canopy cover. Impacts of the thermal environment on nest size could be mediated through two routes: thermoregulation and worker population dynamics. For thermoregulation, a wood ant nest with a small worker population has to rely on direct sun radiation to reach and maintain a sufficient inner nest temperature (Brandt, 1980; Frouz & Finér, 2007). In contrast, thermoregulation of nests with larger worker populations can be independent of sun exposure because of sufficient endogenous heat generation, based on the metabolism and clustering behaviour of workers (Rosengren *et al.*, 1987; Frouz, 2000) and microbial heat production within the nest material (Coenen-Stass *et al.*, 1980; Frouz, 2000). In terms of worker population dynamics, brood development rate and the egg production rate of queens increase with increasing temperature; meanwhile, worker longevity decreases (Porter & Tschinkel, 1993; Asano & Cassill, 2012). The trade-off between brood developmental rate, egg reproductive rate and worker longevity determines how the nest grows in size, which is related to the potential of producing sexual offspring (Tschinkel, 1993).

Our temperature measurements showed that the thermal environment of areas with higher canopy cover was generally colder and more stable than that of more exposed areas (Fig. 4.3). To cope with the cold, nests in shady areas must be large enough to execute effective thermoregulation. Among our sampling points, the maximum canopy cover was 86%. This means that even in the shadier areas nests may sometimes receive sunshine. When sunshine falls on the nest, it might not cover the whole nest mound. This could cause a thermal gradient in the stable cool environment of shadier areas. Therefore, when the sunshine is present, the shadier areas provide a nest with greater variety of thermal environments aiding regulation of worker population dynamics: workers could stay in cooler chambers for longer longevity and could move brood to warmer part for a faster development rate. Shady areas not only necessitate nest growth but could also actively promote it.

For the nests in open areas, although the mean daily minimum temperature is a little lower, the mean daily maximum is much higher than that in shadier areas (Fig. 4.3). The whole or a large part of the nest passively experiences a generally warmer environment, so the nest has less need to grow larger to allow thermoregulation. In addition, warmth-related increases in egg production and brood developmental rate might not be able to compensate for the decreased worker longevity. Thus the nests in very open areas were smaller than those in shady areas. There may also be additional influences from the growth-stage-related thermal requirements for wood ant nests and the forest succession: a newly-built or young wood ant nest is usually small therefore might not survive in shadier areas, and the dynamics of forest succession can result in the canopy modification. Overall, local temperature was probably the primary mediating factor for the relationship we found between canopy cover and nest size. Modelling nest size growth in different thermal environments could be a feasible approach for future studies of red wood ants, with physiological data related to temperature, for example, the relationship of temperature with worker longevity, brood developmental rate and queen’s egg production.

In addition to temperature, food resource availability is another factor which influences wood ant nest size (Sorvari & Hakkarainen, 2005; Punttila & Kilpeläinen, 2009). The majority of the ants in the trails connecting trees and nests are foragers, which collect honeydew from aphids (more than 90% of a colony’s nutrition) (Rosengren & Sundström, 1991; Gordon *et al.*, 1992). Low canopy cover may therefore imply a decrease of available foraging trees for wood ant nests. Our study showed that nests with foraging trails were generally located in shadier areas and nests without foraging trails in more exposed areas. Among the nests with foraging trail/s, minimum foraging trail length was shorter in shady areas than that in open areas, which, as would be expected, indicated that nests were closer to trees in shady areas than in open areas. Nests with foraging trail/s also were larger than nests without foraging trail/s (Fig. 4.4). This matches the findings of a previous study at the same site using a partially overlapping sample set, which also found that *F. lugubris* nests with foraging trail/s were larger and in shaded areas than nests without any foraging trail (Ellis *et al.*, 2014).

Although we might be able to assume that shadier areas provided more possible food resources resulting in the presence of foraging trails, the direction of causality between nest size and the presence of foraging trails is not clear (Fig. 4.7). On the one hand, an established foraging trail may provide more food to promote nest growth. On the other hand, an alternative hypothesis is that only nests above a certain size are able to establish and maintain a lasting foraging trail. Our data showed that although nests with foraging trail/s were on average bigger than those without a trail, the minimum nest size was similar for nests both with and without foraging trails (Fig. 4.4). This would seem to rule out the existence of a nest size threshold which determines whether a nest starts foraging or not, at least within our observed range of nest sizes, and so it is quite possible that the presence of one or more foraging trails promotes increased nest size. Therefore, in addition to local temperature, food resource availability is another possible mediating factor for the relationship between canopy cover and nest size. Interestingly, we only found a few small nests in highly shady areas (for example, over 70% canopy cover, see Fig. 4.1). Food resources are unlikely to be limiting in these areas, so there should be other reasons why small nests are less common. For example, if a new nest in a highly shady area does not grow over a “threshold” size, it may not survive over the winter. It seems that the thermal effects of canopy cover are more important than the relationship with food resource availability, in terms of nest size. We therefore suggest an initial mechanistic process when a nest is newly built: higher canopy cover implies nearer trees resulting in higher food resource availability, and the effects of the thermal environment on worker population dynamics promotes nest growth. The benefits of larger nest size for thermoregulation could result in a positive feedback on nest growth once the nest reaches a certain size. Further work is needed to investigate the relative importance of these different effects over the course of colony establishment, growth and maturity.

At the colony level, we found a trend that total colony size increased with increasing canopy cover. Total colony size was also related to local temperature in the same way. The trend between total colony size and canopy cover was eliminated when a three-way partial correlation was applied to remove the effects of local temperature. These results indicate that, similar to the nest level, local temperature seems to be a mediating factor between canopy cover and total colony size (Fig. 4.7). Furthermore, annual solar radiation had no effect on total colony size nor local temperature in our study; this further supports that the thermal environment experienced by wood ant colonies was strongly determined by canopy cover in this flat area. If higher canopy cover results in increasing total colony size, this could occur in two ways: a polydomous colony has larger total colony size either because it has bigger nests, or because it has more nests, or both. For the first way, we found that a colony that had larger total colony size also had larger mean nest size. This suggests that canopy cover probably influences total colony size through the thermal effects on nest size discussed above. Apart from nest size, our results also showed that total colony size increased when the number of nests increased (Fig. 4.5). For these reasons, we suggest that a polydomous wood ant colony may increase total colony size by both ways: increasing the size of each nest and increasing the number of nests, but mainly by the former. We also suggest that these two approaches compensate for the effects from each other because there was a borderline significant negative trend between the nest size and the number of nest per colony (Fig. 4.6). Moreover, neither canopy cover nor local temperature was related to number of nests per colony (Fig. 4.7). It seems that if the canopy cover has impacts on the qualities of the environment (eg: local temperature or food resource abundance) that affect total colony size, it acts more at the nest level (individual nest size) than at the colony level (the number of nests per colony).

This paper presents a study specifically focused on the relationships of canopy cover to ant nest size and colony size. Our results support and strengthen a marginally significant trend between canopy cover and nest size which was found at the same site by Ellis *et al.* (2014). The stronger finding in our study is probably due to methodological differences. First, Ellis *et al.* (2014) actively chose the largest ten colonies for a nest network study; in our study an even-distribution survey was performed. Second, Ellis *et al.* used images from digital photographs for canopy cover; in our study the circle images of sky which were taken by a fisheye lens provided a complete estimation of canopy cover. Third, colony identity was included in our analyses. Frouz and Finér (2007) also found similar relationships between nest size and canopy cover in another red wood ant species *Formica polyctena*. This study again focussed only on the nest level, and used a semi quantitative scale to estimate shading, which differentiates three levels of shading by daily sunshine hour (Frouz, 2000).

In regard to the canopy-related relationships between polydomy and colony-level organisation, previous studies have showed two different results. Sorvari and Hakkarainen (2005) reported a higher degree of polydomy in *F. aquilonia* in clear-cut areas where the colonies experienced an extreme environment. They hypothesised that new nests are established by budding more frequently in clear-cuts than in forest interior in order to be near the forest edge for food resources. In contrast, Punttila (1996) suggested that monogynous (monodomous) populations of *F. lugubris* should be common in young forest before the canopy closure, whereas polygynous (polydomous) *F. aquilonia* should dominate in older forests and in the interior areas. He suggested a mechanism from inter-specific competition and forest succession: with bigger size of the dispersing females, *F. lugubris* is a more efficient coloniser than is *F. aquilonia*. Female *F. lugubris* disperse to a young forest first where the canopy is still open, and *F. aquilonia* dominates over other species when it comes in the gradually mature forest later by nest budding. Another survey for several mound-building species (including *F. lugubris* and four red wood ant species) was conducted by Punttila and Kilpeläinen (2009) in Finland. They found species-specific associations of nest size with canopy cover. In our study, neither a positive nor negative relationship between canopy cover and the number of nests was found. We suggest that the impacts from canopy cover acts on the nest level rather than on the colony level. This further supports the finding of Ellis *et al.* (2014) which also found no relationship between the number of nests and canopy cover (10 colonies with a total of 140 nests). Overall, the differences between studies may result from the differences between sampling sites and between methodologies, for example, whether other wood ant species are present or not, whether the ants are experiencing normal forest succession or extreme events such as clear-cutting, and whether the studies are focused within or across species.

The most direct approach of understanding the influence of an environmental factor on a species is probably to examine their physiological or life-history characteristics in direct response to the environmental factor, for example, temperature. However, a laboratory approach has some limitations for our question. Investigating the relationship between canopy cover and wood ant nest size in the field solves it in many aspects. First, it is not feasible to simulate the daily or annual temperature in a laboratory approach because the exact dynamics are complex. Canopy cover provides an index for estimating local thermal environment. Second, we can obtain the nest size data in a natural environment with little disturbance to colony function and organisation. Moreover, the present study shows an overall reaction of wood ant nests to canopy cover. Canopy cover may influence nests by changing not only the features of temperature but also the food resource availability (Fig. 4.7). Future studies could involve canopy manipulation or the seasonal variation in canopy cover to monitor the long-term change in nest size and the colony-level organisation on wood ant species, which are ecologically significant in the forest ecosystem. As the effect of climate change on species can act through multiple and complex ways (changes in vegetation, species interaction and human activity), species-specific responses to future climate change are challenging to predict. A prediction based on causal relationships between the environments (eg: canopy cover) and the life-history characteristics (eg: nest size and colony size) may suggest possible future outcomes, thus help species' conservation and potentially reduce negative impacts of climate change on these species.

# Acknowledgements

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# Chapter 5 – Is Bergmann's rule applicable to the relationship between altitude and ant colony size? Geographic gradient versus microclimate

## Abstract

Bergmann's rule is a common eco-geographic rule which describes the increase of body size from tropical to polar latitude. Temperature is the most likely driving force of Bergmann's rule, so the latitudinal relationship can also be applied to altitudinal gradients. The colony size of social insects correlates with many physiological and behavioural features of the colony, and therefore could be biologically analogous to the body of a unitary organism. Combining these ideas, this study focused on the application of Bergmann's rule to the relationship between altitude and ant colony size. In addition to the large-scale geographic gradients, small-scale factors such as microclimate also have impacts on environmental conditions. We investigated the relationship between altitude (geographic gradient), canopy cover (an index of microclimate) and total colony size in *Formica lugubris* and *F. paralugubris* in the Swiss Alps. Polydomy was also considered in analyses. Local temperature data were recorded across different altitudes and canopy cover. Total colony size of these two red wood ant species increases with increasing canopy cover. Total colony size also follows Bergmann's rule along altitude when canopy cover is controlled for. The small-scale factors such as microclimate may be more significant than the large-scale geographic factors in determining a life-history characteristic of an animal. We therefore suggest that studies of eco-geographic trends should also consider the small-scale factors. This study can be a reference for future conservation actions concerning red wood ant species.

# Introduction

Habitat, lifestyle, and life history of a given animal are correlated (Stearns, 1992). Body size is one of the most significant life-history characteristics because it is associated with most aspects of biology, including physiological performance and other characteristics (Calder, 1996; Brown & Lomolino, 1998; Chown & Gaston, 2010). Bergmann's rule is a common eco-geographic rule of life-history characteristics: the increase of body size from tropical to polar latitudes (Bergmann, 1847; Mayr, 1956; Watt *et al.*, 2010). According to the excerpts translated by James (1970), Bergmann's rule has linked temperature to latitude because temperature on a global scale decreases from the equator to the poles. The original Bergmann's rule and the redefinition proposed to apply the rule inter-specifically (James, 1970; Blackburn *et al.*, 1999; Watt *et al.*, 2010). However, Bergmann attempted to test his rule among races of domestic animals (Watt *et al.*, 2010). Some researchers also state that Bergmann's rule could be used to describe the pattern of a species' body size change along latitude (James, 1970; Angilletta *et al.*, 2004a). Meiri (2011) suggests that the rule is a pattern that can be studied at any taxonomic level and in any taxon. Therefore, Bergmann's rule is used more loosely to describe a trend of the body size increases with rising latitude or with the decline of temperature either intra-specifically or inter-specifically.

Bergmann originally suggested applying his rule to homeotherms (endotherms) (James, 1970; Watt *et al.*, 2010). More than 65% of endotherms show Bergmann's cline in body size (i.e. body size increases with rising latitude) (Ashton *et al.*, 2000; Ashton, 2002; Meiri & Dayan, 2003). The fasting endurance hypothesis (also known as starvation resistance) has been considered to be the most likely hypothesis of the driver for endotherms: larger body size increases survival during the longer resourceshortage season in higher latitude as a result of the relatively larger energy reserves and lower metabolic requirements per unit weight (Lindstedt & Boyce, 1985; Millar & Hickling, 1990). Although the original focus was on endotherms, the suggested revisions of Bergmann's rule by Paterson (1990) and Blackburn *et al.* (1999) do not exclude ectotherms (reviewed by Watt *et al.*, 2010). A review study by Vinarski (2014) shows that there is not a universal pattern in the geographic variation of body size for each large taxon of ectotherms (e.g. molluscs, arthropods, amphibians, reptiles, etc.). Both adaptive and non-adaptive hypotheses arise to explain Bergmann's cline for ectotherms (see Chapter 1). The non-adaptive hypothesis illustrates how thermal effects on biochemical processes can result in the temperature-size relationship: a smaller final body size of ectotherms is produced at increased rearing temperature (Atkinson, 1994; Angilletta & Dunham, 2003; Angilletta *et al.*, 2004b). The adaptive hypothesis considers the costs and benefits of a given life history to describe the reason that natural selection promotes genotypes which grow more slowly but mature at a larger size when raised at lower temperatures (Atkinson & Sibly, 1997; Angilletta *et al.*, 2004b).

In social insects, the individual and the colony are two levels of organisation (Kaspari & Vargo, 1995; Tschinkel, 1999; Clémencet & Doums, 2007). The allometry or the biological scaling should be applied on both levels of organisation. The colonies can also be characterised by their physical and numerical features (Tschinkel, 1991). The total number of individuals or the worker population in a colony has been used to represent the colony size (Kaspari & Vargo, 1995; Tschinkel, 1998, 1999). Some researchers have suggested that the colony of social insects could be biologically analogous to the body of a unitary organism (Tschinkel, 1991; Kaspari & Vargo, 1995; Tschinkel, 1998, 1999; Hölldobler & Wilson, 2009; Lanan *et al.*, 2011). Basic features of the physiology (ie: metabolic rate) and life history (ie: growth) of whole colonies follow virtually the same size dependences as unitary insects (Hou *et al.*, 2010). Just as body size has a defining role for solitary organisms, colony size correlates with many features of a social insect colony, including competitive abilities, foraging behaviours and life span (O'Donnell & Jeanne, 1992; Pamilo *et al.*, 1992; Eckert *et al.*, 1994; Herbers & Choiniere, 1996; Palmer, 2004). Some studies therefore have focused on Bergmann's rule on colony size (Table 5.1).

**Table 5.1.** Summary of previous research on Bergmann's rule in social insects, broken down into inter-specific or intra-specific Bergmann's rule between latitude (or altitude) and body size (or colony size) of social insects. The boxes marked "?" lack data and are the focus of this study. Reversed relationship (converse Bergmann's cline) between latitude (or altitude) and body size (or colony size) were not found in inter-specific nor intra-specific studies.

| | | | | Support | No relationship |
|---|---|---|---|---|---|
| Bergmann's rule | Inter-specific | Latitude | Body size | [1][2] | [3] |
| | | | Colony size | [4] | [2][3][5] |
| | | Altitude | Body size | None found | [3] |
| | | | Colony size | None found | [3] |
| | Intra-specific | Latitude | Body size | [6][7][8] | None found |
| | | | Colony size | None found | [8] |
| | | Altitude | Body size | [8][a][9][b] | None found |
| | | | Colony size | ? | ? |

[1] Cushman *et al.* (1993); [2] Kaspari (2005); [3] Geraghty *et al.* (2007); [4] Kaspari and Vargo (1995); [5] Porter and Hawkins (2001); [6] Daly *et al.* (1991); [7] Rust (2006); [8] Heinze *et al.* (2003); [9] Stone (1993); [a] two high altitudinal populations in the study were larger than expected from latitude; [b] a study of solitary bees.

The decline of temperatures with rising altitude and latitude is the main comparable similarity between altitude and latitude (Brown & Lomolino, 1998). If Bergmann's rule is driven by temperature, the same relationship along latitude could be applied to altitudinal gradients, as the most important similarity between altitude and latitude is the decline of temperatures with rising altitude and latitude (Brown & Lomolino, 1998). Social insect colony size can be considered as analogous to body size. For these reasons we ask, can Bergmann's rule be applied to the relationship between altitude and colony size? Understanding the relationship between altitude and colony size is important for conservation actions because climate change has different impacts along altitudinal gradients (Parmesan, 2006). There have been some studies on inter-specific or intra-specific Bergmann's rule between latitude (or altitude) and body size (or colony size) of social insects, but no intra-specific investigation for Bergmann's rule between altitude and colony size (Table 5.1). However, an inter-specific study showed that evolutionary constraints and phylogeny have greater influence on colony size and body size than altitudinal or latitude gradients do (Geraghty *et al.*, 2007). A review by Shelomi (2012) also indicates that inter-specific studies found Bergmann's cline less frequently than intra-specific studies did. The outcome results from the variation among species within the examined clades. Therefore, an intra-specific research is probably more feasible to reveal the eco-geographic mechanism on colony size.

In addition to large-scale geographic gradients such as altitude, small-scale factors have impacts on physical conditions of the environment. Microclimate is the set of climatic conditions which are measured in local areas near the ground surface (Chen *et al.*, 1999; Geiger *et al.*, 2009). For terrestrial ecosystems, habitat type (e.g. grassland and woodland) is a major modifier of the microclimate experienced by organisms, for example, it affects the extreme value of temperatures (Suggitt *et al.*, 2011). Taking woodland habitat for example, microclimatic variables, especially solar radiation, local air temperature (air temperature at the ground surface) and soil temperature, are highly sensitive to the changes of canopy (Chen & Franklin, 1997). Spatial heterogeneity of woodland is commonly and significantly caused by small canopy openings (Clinton, 2003). The canopy features therefore contribute to structural complexity and provide high spatial and temporal variability on the forest floor within woodland habitat (Chen & Franklin, 1997; Chen *et al.*, 1999). Moreover, canopy cover may be the most obvious and significant small-scale factor which is associated with altitudinal gradients. Canopy cover is also known to affect ant's nest size (Chen & Robinson, 2014; Ellis *et al.*, 2014), distribution (Punttila, 1996) and reproduction (Sorvari & Hakkarainen, 2005). Since the most likely main driving force of Bergmann's rule, temperature, is also related to canopy cover (Rodriguez-Garcia *et al.*, 2011; Suggitt *et al.*, 2011; Huang *et al.*, 2014), we also investigated the effects of canopy cover together with altitude gradients aiming to see how altitude affects colony size when accounting for canopy cover.

For some ant species, one colony may settle in either one nest or several spatially separated but socially connected nests (polydomy) (Hölldobler & Wilson, 1977; Ellis & Robinson, 2014; Robinson, 2014). Debout *et al.* (2007) define polydomy as "an arrangement of an ant colony in at least two spatially separated nests", and the separated distance of these two nests should be obviously larger than that of two nest chambers in the core nest structure. A polydomous colony may have multiple ecological benefits such as risk spreading (Debout *et al.*, 2007; Robinson, 2014) and both resource discovery (Cook *et al.*, 2013) and exploitation (by establishing new nests near food resource) (Lanan *et al.*, 2011). Being polydomous may also allow colonies to overcome the constraints on increasing size for monodomous colonies (Robinson, 2014), for example, increasing total colony size through increasing nest number. Therefore, domy form (whether monodomous or polydomous) of the colony is also a factor of interest within an investigation focusing on Bergmann's rule and colony size.

In this study, we investigate the relationship between altitude, canopy cover and both nest size (worker population in a nest) and total colony size intra-specifically in polydomous populations of Eurasian red wood ants *Formica lugubris* and *Formica paralugubris*. Both species have ecologically significant impacts on forest ecosystem (Sudd & Lodhi, 1981; Punttila *et al.*, 2004). Among the red wood ant group, *F. lugubris* and *F. paralugubris* are of interest for altitudinal studies because they have been recorded at altitudes from 800 to 2400 metres in Central Europe (Kutter, 1965; cited by Sudd *et al.*, 1977) and from 1100 to 2000 metres (Bernasconi *et al.*, 2006; Glaser, 2006), respectively. We also checked the effects of canopy cover and altitude on local temperature by collecting thermal data at the colony locations. Larger colonies may cope better with colder environments due to better starvation resistance capability (Kaspari & Vargo, 1995) or increased abilities to regulate inner nest temperature (Rosengren *et al.*, 1987; Punttila & Kilpeläinen, 2009). Since thermal environments are colder both with rising altitude and canopy cover, we predict positive relationships between nest/colony size and altitude, and between nest/colony size and canopy cover.

## Materials and Methods

### Species and Location

To have a large range of altitude, we conducted our study in a part of the Alps (46°15'16"-46°20'23"N, 07°02'35"-07°09'39"E) in Ollon, Vaud, Switzerland with an altitudinal range of 800-2100 m. There is a transition of Scots pine *Pinus sylvestris*-dominated forests to Swiss Pine *Pinus cembra*-dominated stands from 1200 to 2400 m in the Swiss Central Alps, with a transition zone between 1600 and 2000 m (Hättenschwiler & Körner, 1995). The study species were two red wood ant sibling species *Formica lugubris* and *Formica paralugubris* (Hymenoptera: Formicidae) (Seifert, 1996). These two species are highly similar to each other in the aspects of morphology and ecology, and have to be morphologically discriminated from each other under a stereo-microscope (Seifert, 1996). Both *F. lugubris* and *F. paralugubris* form polydomous colonies in the Swiss Alps, although *F. lugubris* has monodomous social organisation in other parts of its geographical range (Bernasconi *et al.*, 2005; Maeder *et al.*, 2005; Ellis & Robinson, 2014). We defined a polydomous colony as a group of nests which are connected to each other by trails, with trails defined as a distinct path with at least 10 workers in 40 cm (Chen & Robinson, 2014; Ellis *et al.*, 2014). The term "altitude" is the vertical distance between an object and a reference point (McVicar & Körner, 2013). In this study, altitude is used to indicate the vertical distance from sea level.

## Methods

The study was performed in 2013, from late June, when canopy cover had reached a relatively stable level, to early September, when the air temperature started to decrease. To make sure that our sampling areas were occupied by *F. lugubris* and *F. paralugubris*, we conducted a 10-km-by-4-km- transect to include the nests recorded by a previous survey (D. Cherix, personal communication). The transect primarily covered the road "Route du Col de la Croix" from Villars-sur-Ollon to Les Diablerets, and was divided into sections every 200 metres. We accessed each section to find our target species mainly through roads, paths and trails. Apart from private and inaccessible areas, the survey for each section focused on woodlands, deep forests and the edges between woodlands and pastures. In the Swiss Alps, several wood ant species (eg: *F. rufa*, *F. pratensis*, *F. lugubris* and *F. paralugubris*, etc.) have overlapping distributions. According to morphological differences of workers, we identified colonies of *F. lugubris* and *F. paralugubris* as distinct from other ant species in the field. Because the two study species had to be distinguished morphologically by stereo-microscope, fifty workers were collected from each nest for confirming species identification.

We defined a distinct trail (with at least 10 workers in 40 cm) from a nest to a tree as a foraging trail (Chen & Robinson, 2014), although this does not mean that the nests without any foraging trails were not foraging at all. The location detail (latitude and longitude) of each colony was recorded. We recorded and mapped the spatial distribution pattern of nests of each colony, including the trails between nests, and foraging trails between nests and trees. A Mound-Volume method was used to estimate nest size (Chen & Robinson, 2013, 2014; Ellis *et al.*, 2014); two diameters and the height (cm) of nest mound were multiplied to represent the worker population of each mound-building wood ants nest. We took a photo skyward above each nest using 180-degree hemispherical lens (FC-E8 fisheye lens with Coolpix 5000, Nikon Corporation, Tokyo, Japan), which produces circular images that record the size, shape, and location of gaps of the canopy. Canopy cover (percentage) was estimated from the circular photo using the software Gap Light Analyzer 2.0 (Frazer *et al.*, 1999; Chen & Robinson, 2014).

To investigate how the small-scale thermal environment of the nest is influenced by altitude and canopy cover, we chose a subsample of nests to record local temperatures. For this purpose, we divided altitude and canopy cover into three (< 1500m, 1500-1800m, > 1800m) and two (< 60%, > 60%) categories, respectively, according to the altitude and canopy cover data from preliminary survey. Five nests belonging to each combination of categories (six combinations) were chosen, giving a total of 30 nests. A temperature-recording device was fixed on the ground right next to the north side of each chosen nest. The device was made up of a polyethylene terephthalate (PET) tube (diameter = 10 cm, length = 20 cm) wrapped in aluminium foil to reduce the effect of solar radiation (Suggitt *et al.*, 2011; Chen & Robinson, 2014). Hourly environmental temperatures were recorded by the thermal data logger (iButton: DS1921G-F5; Maxim/Dallas Semiconductor, TX, US) within each device during the period from mid-July to early-September.

## Statistical Analyses

Total colony size was calculated as the sum of sizes of each nest belonging to a polydomous colony to represent the total worker population in a colony. Size data (nest size, total colony size and mean nest size) were transformed by $\log_{10}$ to normalize the distributions. We used “lmer” function from the “lme4” package of R (version 3.1.0, R Development Core Team) to fit linear mixed-effects model for the effects of domy form (whether monodomous or polydomous), altitude and canopy cover on nest size. Colony identity was included as a random effect in the model. The “anova” function and AIC value were used for model selection.

Linear mixed-effects models were also used for the effects of altitude and canopy cover on local temperature measurements at nesting locations: local daily mean temperature ($\mathrm{Mean}_{\mathrm{Local}}$), local daily maximum temperature ($\mathrm{Maximum}_{\mathrm{Local}}$) and local daily minimum temperature ($\mathrm{Minimum}_{\mathrm{Local}}$). The identity of the colony to which the recording device belonged to was included in the model as a random effect. As background values, air temperature measurements ($\mathrm{Mean}_{\mathrm{Air}}$: daily mean; $\mathrm{Maximum}_{\mathrm{Air}}$: daily maximum; and $\mathrm{Minimum}_{\mathrm{Air}}$: daily minimum) of the days which we recorded local temperatures were included respectively in the models as covariate factors to control the changes of daily measurements for the analyses of local temperatures. We obtained air temperature data from the weather station "Gerance Service SA IVAUDVIL3" (46°17'52.8"N 7°03'18.0"E; 1249 m; from Weather Underground, http://www.wunderground.com/), which is approximately 1.5 km from the transect.

We used a linear regression model for the effects of domy form, canopy cover and altitude on total colony size and mean nest size. The "anova" function of R package was used for model selection. We used Kruskal-Wallis rank sum test for the differences of mean canopy cover, altitude, total colony size and mean nest size between colonies with different number of nests. The "kruskalmc" function from the "pgirmess" package of R was used for multiple comparison test after Kruskal-Wallis' test, if necessary. Linear regression model and Kruskal-Wallis' test were conducted with R statistics package (version 3.1.0, R Development Core Team).

## Results

### Nest size, colony size, canopy cover and altitude

One hundred and fifty nine colonies of *F. lugubris* with a total of 286 nests, and 94 colonies of *F. paralugubris* with a total of 140 nests were sampled and recorded for this study. We found that nest size increased significantly with increasing canopy cover, and with increasing altitude (Model 6 in Table 5.2, Figs 5.1a and 5.1b). There was a significant negative interaction effect between canopy cover and altitude on nest size (Model 6 in Table 5.2, Fig. 5.1c). Monodomous colonies had significantly larger nest size than polydomous colonies did (Model 6 in Table 5.2, Fig. 5.1d). The species showed no significant difference in nest size (Models 1 to 5 in Table 5.2). The species identity factor did not significantly improve the model (comparison between Models 5 and 6 in Table 5.2, $\chi^2 = 0.49$, $P = 0.48$), we therefore chose the reduced model (Model 6 in Table 5.2, with domy form, canopy cover, altitude and the interaction between canopy cover and altitude) as the best model.

Total colony size, which was the sum of sizes of all nests in that colony, significantly increased with increasing mean canopy cover across all nests of that colony and with increasing altitude (Model 6 in Table 5.3, Figs 5.2a and 5.2b). The interaction of mean canopy cover and altitude had a significant negative effect on total colony size (Model 6 in Table 5.3, Fig. 5.2c). Total colony size of polydomous colonies was significantly larger than that of monodomous colonies (Model 6 in Table 5.3, Fig. 5.2d). The species identity factor had no significant effect on total colony size (Models 1 to 5 in Table 5.3), and did not significantly improve the model (ANOVA test for Models 5 and 6 in Table 5.3, $F = 0.26$, $P = 0.61$). The reduced model (Model 6 in Table 5.3, with domy form, canopy cover, altitude and the interaction between canopy cover and altitude) was therefore chosen as the best model. Relationship between mean nest size, domy form, canopy cover and altitude are provided as appendices (Table 5.S1). Canopy cover at our 426 sampled nests ranged from 19% to 96%, with a median of 80%, and altitude of nests ranged from 1247 m to 1995 m, with a median of 1660 m.

**Table 5.2.** The relationships between $\log_{10}$ nest size, canopy cover, altitude and domy form. Data were fitted with linear mixed-effects model (253 colonies with 426 nests). The statistical baseline of "Species" factor was set as *Formica lugubris*. The statistical baseline of "Domy" factor was set as monodomy in Models 5 and 6. The "Interaction" term mean the interaction effect between canopy cover and altitude factors. The "anova" function of R package and AIC value were used for model selection. Model 5 was significantly better than Models 1 to 4 ($\chi^2$ = 37.87-103.29, $P < 0.001$). There was no significant difference between Models 5 and 6 ($\chi^2$ = 0.49, $P$ = 0.48). Due to very similar AIC values of Model 5 and Model 6, the reduced model (Model 6) was chosen as the best model.

| | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|
| | Estimate | *t* value | Estimate | *t* value | Estimate | *t* value |
| Intercept | 4.04e+00 | 23.34*** | 6.59e+00 | 16.46*** | 5.69e+00 | 83.52*** |
| Canopy Cover | 1.88e-02 | 8.40*** | - | - | - | - |
| Altitude | - | - | -6.98e-04 | -2.88** | - | - |
| Domy | - | - | - | - | -4.17e-01 | -5.21*** |
| Species | 1.21e-01 | 1.60 | 1.10e-01 | 1.28 | 5.64e-02 | 0.50 |
| AIC | 909.25 | | 966.38 | | 948.54 | |

| | Model 4 | | Model 5 | | Model 6 | |
|---|---|---|---|---|---|---|
| | Estimate | *t* value | Estimate | *t* value | Estimate | *t* value |
| Intercept | -1.29e+00 | -0.67 | -1.05e+00 | -0.58 | -1.14e+00 | -0.63 |
| Canopy Cover | 7.93e-02 | 3.32*** | 7.78e-02 | 3.44*** | 7.92e-02 | 3.52*** |
| Altitude | 2.97e-03 | 2.75** | 2.95e-03 | 2.88** | 3.01e-03 | 2.96** |
| Interaction | -3.35e-05 | -2.47* | -3.21e-05 | -2.49* | -3.29e-05 | -2.57* |
| Domy | - | - | -4.51e-01 | -6.32*** | -4.57e-01 | -6.47*** |
| Species | 1.12e-01 | 1.41 | 5.21e-02 | 0.70 | - | - |
| AIC | 904.96 | | 869.09 | | 867.59 | |

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

**Table 5.3.** The relationships between $\log_{10}$ total colony size, canopy cover, altitude and domy form. Data were fitted with linear model. The statistical baseline of “Species” factor was set as *Formica lugubris*. The statistical baseline of “Domy” factor was set as monodomy in Models 5 and 6. The “Interaction” term mean the interaction effect between canopy cover and altitude factors. The “anova” function of R package was used for model selection. Model 5 was significantly better than Models 1 to 4 ($F$ = 7.71-35.57, $P < 0.01$). There was no significant difference between Model 5 and Model 6 ($F = 0.26$, $P = 0.61$), the reduced model (Model 6) was therefore chosen as the best model.

| | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|
| | Estimate | *t* value | Estimate | *t* value | Estimate | *t* value |
| Intercept | 4.38e+00 | 28.44*** | 6.86e+00 | 19.48*** | 5.69e+00 | 100.90*** |
| Canopy Cover | 1.90e-02 | 9.47*** | - | - | - | - |
| Altitude | - | - | -6.45e-04 | -3.06** | - | - |
| Domy | - | - | - | - | 2.36e-01 | 3.08** |
| Species | 1.74e-02 | 0.26 | 9.54e-03 | 0.13 | 4.42e-02 | 0.58 |
| $F$ | $F_{2, 250} = 44.86$*** | | $F_{2, 250} = 4.71$** | | $F_{2, 250} = 4.79$** | |
| $r^2$ | 0.26 | | 0.04 | | 0.03 | |

| | Model 4 | | Model 5 | | Model 6 | |
|---|---|---|---|---|---|---|
| | Estimate | *t* value | Estimate | *t* value | Estimate | *t* value |
| Intercept | -6.91e-01 | -0.43 | -7.87e-01 | -0.50 | -8.18e-01 | -0.52 |
| Canopy Cover | 7.51e-02 | 3.77*** | 7.62e-02 | 3.90*** | 7.69e-02 | 3.95*** |
| Altitude | 3.79e-03 | 3.13** | 2.81e-03 | 3.22** | 2.83e-03 | 3.25** |
| Interaction | -3.04e-05 | -2.69** | -3.13e-05 | -2.83** | -3.17e-05 | -2.87** |
| Domy | - | - | 2.14e-01 | 3.30** | 2.10e-01 | 3.27** |
| Species | 1.12e-02 | -0.17 | 3.29e-02 | 0.57 | - | - |
| $F$ | $F_{4, 248} = 26.24$*** | | $F_{5, 247} = 24.01$*** | | $F_{4, 248} = 30.04$*** | |
| $r^2$ | 0.29 | | 0.33 | | 0.33 | |

**$P < 0.01$; ***$P < 0.001$

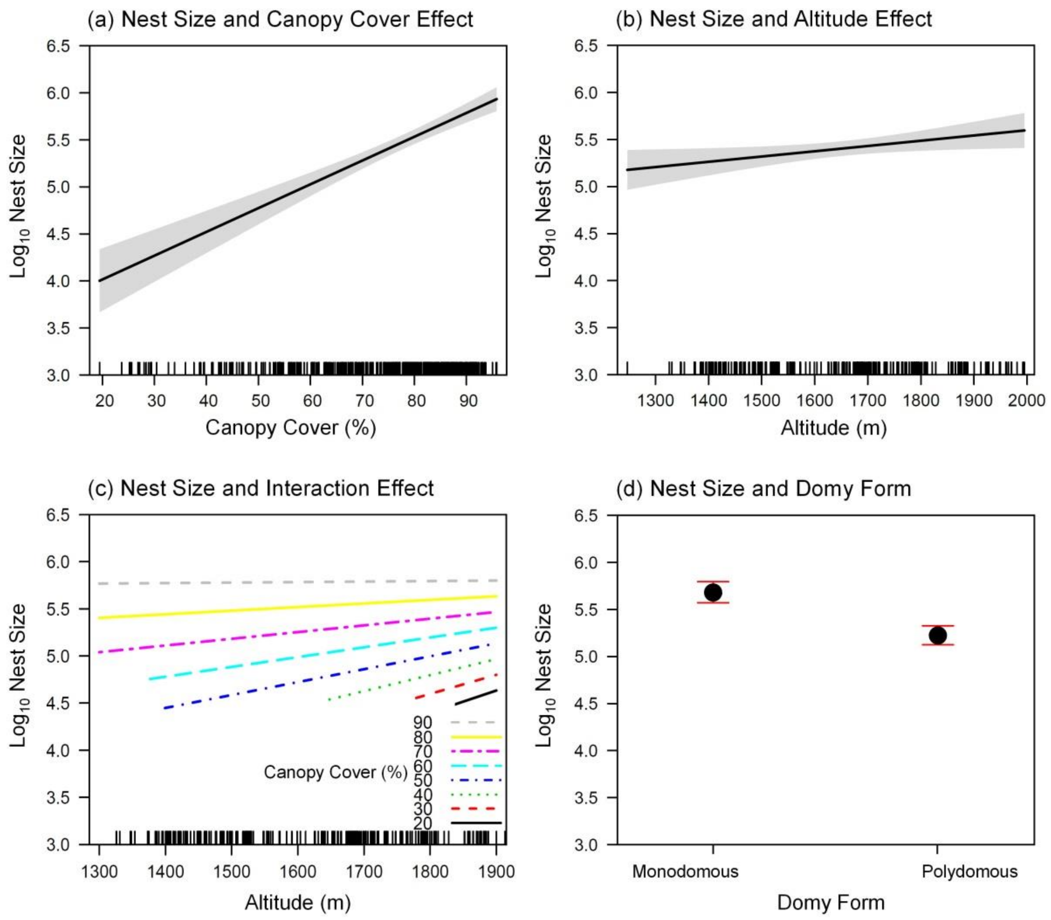

**Figure 5.1.** The relationship between log$_{10}$ nest size, canopy cover, altitude and domy form (effect plots for the fixed effects of linear mixed-effects model, referring to Model 6 in Table 5.2). Grey areas in 5.1a and 5.1b indicate the 95% confidence intervals. Lines of different colours in 5.1c show the relationships between altitude and nest size under different canopy cover. Error bars in 5.1d indicate 1SE.

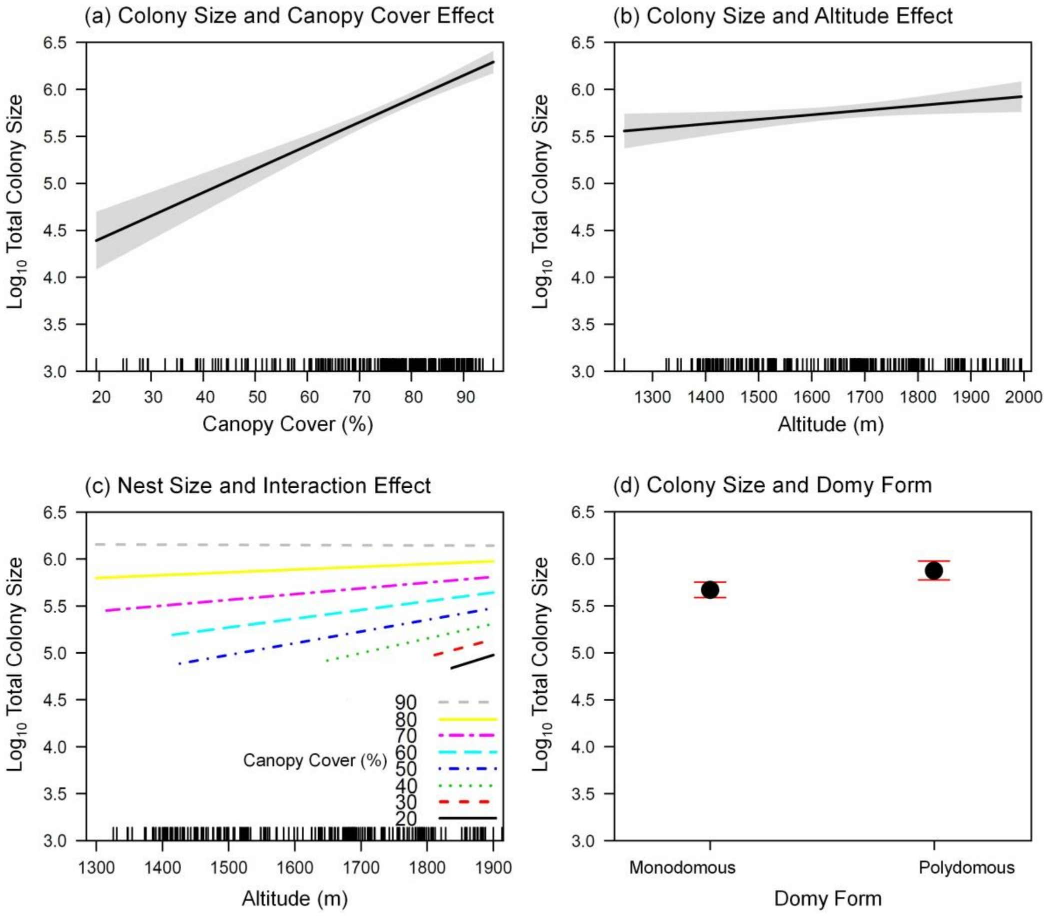

**Figure 5.2.** The relationship between $\log_{10}$ total colony size, canopy cover, altitude and domy form (effect plots for the effects of regression model, referring to Model 6 in Table 5.3). Grey areas in 5.2a and 5.2b indicate the 95% confidence intervals. Lines of different colours in 5.2c show the relationships between altitude and total colony size under different canopy cover. Error bars in 5.2d indicate 1SE.

## Local temperature measurements

Sixteen temperature-recording devices were lost or broken or dislocated in the field. According to the records of the 14 nests from which data loggers were retrieved (one to five nests belong to each combination of categories), daily mean of local environmental temperatures ($\text{Mean}_{\text{Local}}$) was lower with increasing altitude, whereas canopy cover did not affect Mean$_{Local}$ (Fig. 5.3a, Table 5.4). In contrast, daily maximum of local temperatures (Maximum$_{Local}$) were higher in open areas than in shadier areas, whereas altitude had no significant effect on Maximum$_{Local}$ (Fig. 5.3b, Table 5.4). Daily minimum of local temperatures (Minimum$_{Local}$) increased with rising canopy cover, and decreased with increasing altitude (Figs 5.3c and 5.3d, Table 5.4). Local temperature data were recorded for 12-37 days (Mean = 25 days) among these 14 nests, across a range of canopy cover of 28-88% and an altitudinal range of 1373-1981 m.

**Table 5.4.** The relationships between local temperature measurements, canopy cover and altitude, with air temperature measurements as co-variances (Mean$_{Local}$: local daily mean temperatures; Maximum$_{Local}$: local daily maximum temperatures; Minimum$_{Local}$: local daily minimum temperatures; Mean$_{Air}$: air daily mean temperatures; Maximum$_{Air}$: air daily maximum temperatures; Minimum$_{Air}$: air daily minimum temperatures; data fitted by linear mixed-effects model with recorded location as a random effect).

| | | Local Temperature Measurements | | | | | |
|---|---|---|---|---|---|---|---|
| | | Mean$_{Local}$ | | Maximum$_{Local}$ | | Minimum$_{Local}$ | |
| | | Estimate | *t* value | Estimate | *t* value | Estimate | *t* value |
| Main Effects | Intercept | 7.77e+00 | 3.67** | 8.14e+00 | 0.85 | 7.75e+00 | 2.44* |
| | Canopy Cover | -1.14e-02 | -1.00 | -1.22e-01 | -2.38* | 4.05e-02 | 2.37* |
| | Altitude | -4.95e-03 | -5.19*** | -2.52e-03 | -0.58 | -7.42e-03 | -5.14*** |
| Covariates | Mean$_{Air}$ | 9.16e-01 | 51.44*** | | | | |
| | Maximum$_{Air}$ | | | 1.31e+00 | 24.09*** | | |
| | Minimum$_{Air}$ | | | | | 8.72e-01 | 63.14*** |

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

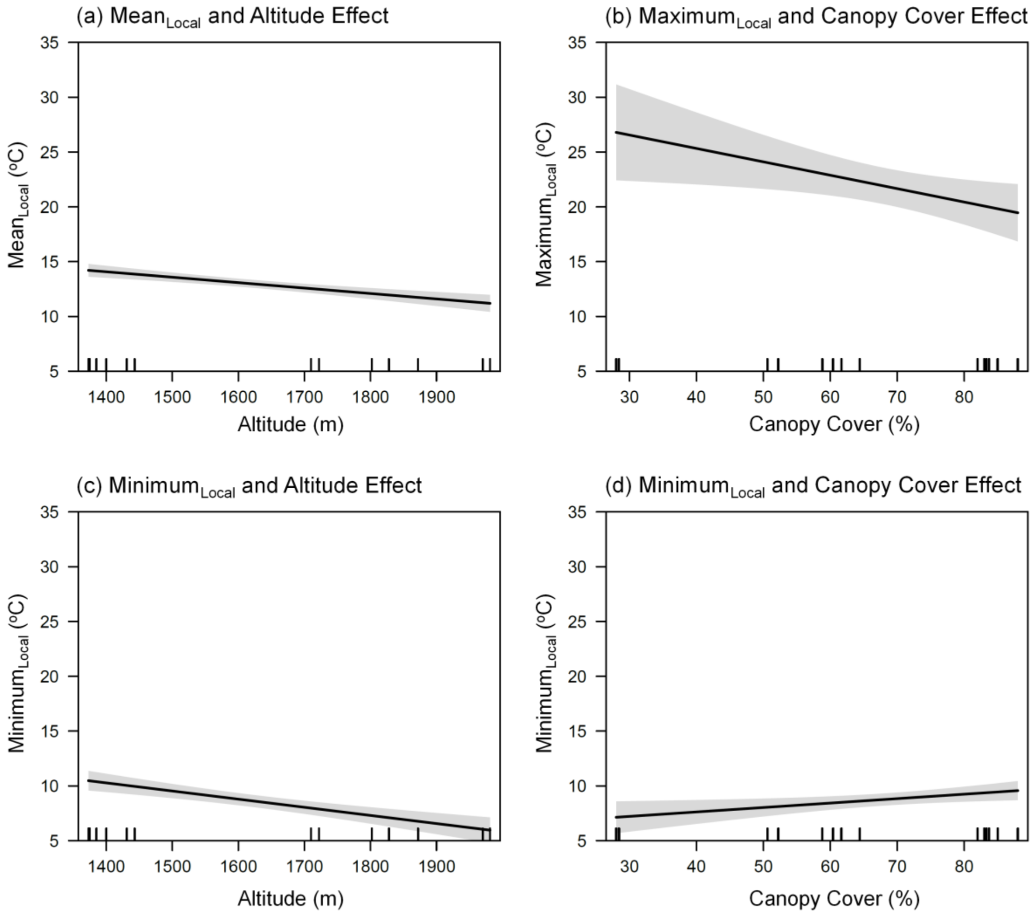

**Figure 5.3.** The relationships between local temperature measurements, altitude and canopy cover (Mean$_{Local}$: local daily mean temperature; Maximum$_{Local}$: local daily maximum temperature; and Minimum$_{Local}$: local daily minimum temperature; effect plots for the fixed effects of linear mixed-effects model, referring to Table 5.4). Grey areas indicate the 95% confidence intervals.

## Colony-level organisation

There was no significant difference between two species in total colony size (Table 5.2 and *t* test: $t = 0.24$, $P = 0.81$) nor in mean nest size (Table 5.S1 and *t* test: $t = 1.07$, $P = 0.29$). Mean canopy cover (*t* test, $t = 0.04$, $P = 0.97$) and altitude (*t* test, $t = -0.60$, *P* = 0.55) of colony locations were not significantly different between two species. We therefore combined the data from the two species for the following analyses. Colonies with different number of nests had different total colony size (Kruskal-Wallis' test, $\chi^2$ = 30.58, *P* < 0.001, see Fig. 5.4 for sample sizes and results of multiple comparison test); colonies with one and two nests tended to have smaller total colony size than those with three and four nests did. Colonies with more nests tended to have smaller variances in total colony size (Levene's test, *F* = 3.10, *P* < 0.01, see Fig. 5.4 for sample sizes); colonies with four and seven nests had smaller variances on total colony size than other colonies did. The number of nests per colony showed no significant relationship with mean canopy cover ($\chi^2$ = 3.85, *P* = 0.70), altitude ($\chi^2$ = 2.30, *P* = 0.89), nor mean nest size ($\chi^2$ = 11.03, *P* = 0.09).

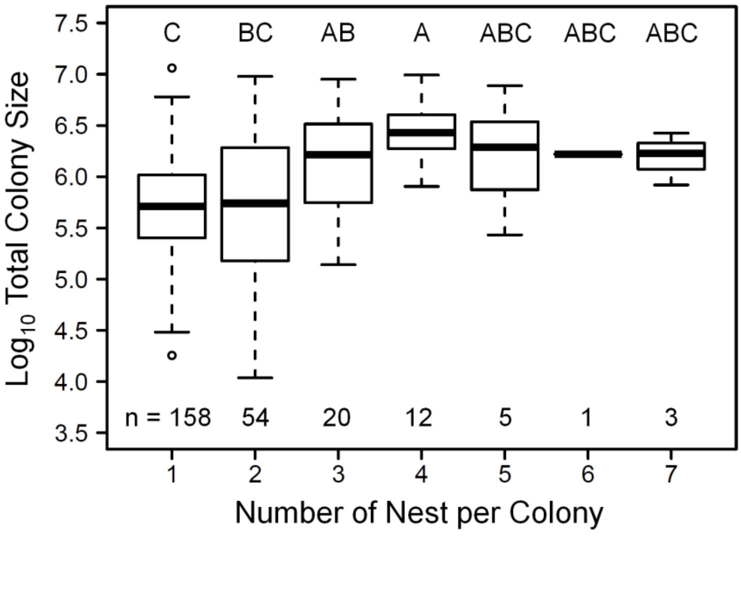

**Figure 5.4.** The relationship between total colony size and the number of nests per colony. Different letters indicate significant difference among groups (Kruskal-Wallis' test and multiple comparison test). Boxplots show the range, quartiles, median and outliers of the data.

# Discussion

Our results reveal that nest size and total colony size (summed size of all nests) of *Formica lugubris* and *F. paralugubris* follow Bergmann's rule along altitude when canopy cover is controlled for (Figs 5.1, 5.2 and 5.5). Among the colonies we sampled and recorded, 62.5% of them are monodomous colonies (*F. lugubris*: 58.5%; *F. paralugubris*: 69.1%). High proportions of monodomous colonies are probably the main reason that we found very similar results for nest size and colony size in the effects of altitude and canopy cover (Table 5.2 and 5.3): colony size data are mainly represented by nest size data.

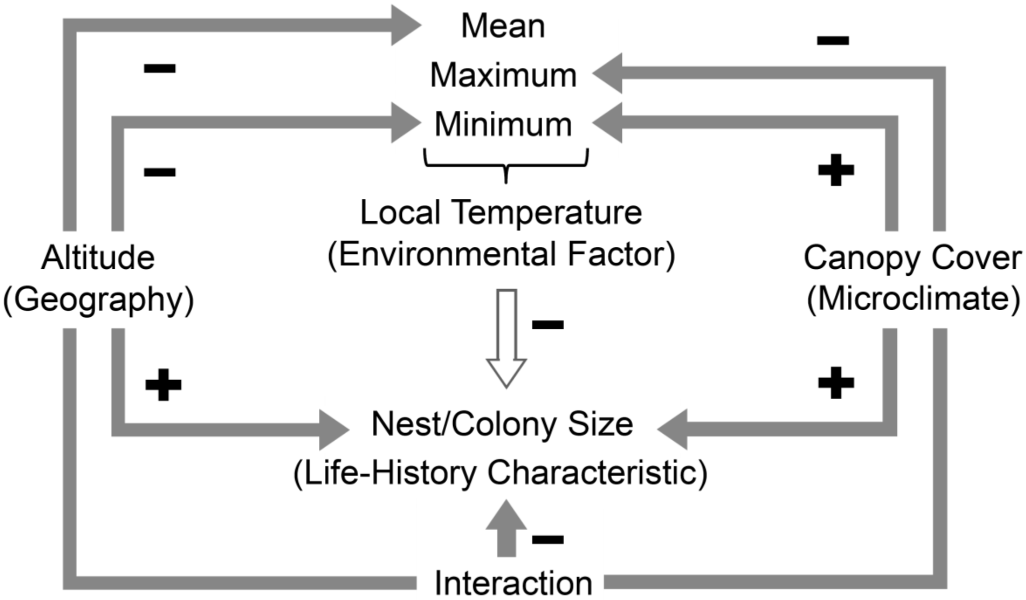

**Figure 5.5.** The relationships of altitude, canopy cover and nest size (colony size) to possible related factors in our study. Arrows illustrate the proposed direction of causality. Solid arrow and hollow arrow indicate the statistically significant and the suggested relationships respectively. Plus and minus signs indicate the relationships as positive and negative respectively. "Interaction" makes reference to the interaction effect between altitude and canopy cover.

As one of the indices of the patterns of vegetation, canopy cover should be considered for a study of altitudinal effects. In fact, there was a negative relationship between altitude and canopy cover in our study (supporting information: Fig 5.S1). Several of our results also show that the effect of canopy cover on nest size and colony size is more powerful than that of altitude. First, when altitude was the only factor added into models (Models 2 in Table 5.2 and Table 5.3), there were negative altitudinal effects on nest size and colony size. These relationships probably resulted from the decrease of canopy cover with increasing altitude (Fig. 5.S1). Second, variations of nest size and colony size along the change of canopy cover were greater than along the altitudinal gradient even when these two factors were added into models simultaneously (Figs 5.1a,b and 5.2a,b). Moreover, our models predict that the slope of the relationship between altitude and nest size or colony size decreases, even to becoming flat when canopy cover is at a high level (ie: with a 90% canopy cover; Figs 5.1c and 5.2c). This means that altitude affects nest size and colony size when the canopy cover is low; meanwhile, nest size and colony size increase in very shady areas irrespective of the altitude at which they are located. These results suggest that the small-scale factors such as microclimate or microhabitat could be more significant than the large-scale geographic factors in determining a life-history characteristic of an animal. A review study for Bergmann’s rule shows nearly equal numbers of Bergmann and converse-Bergmann clines overall (Shelomi, 2012). We propose a possible explanation that some clines (or the absence of a cline) found in previous studies were very likely caused or influenced by small-scale factors rather than the geographic gradient, altitude, which is of interests to the researchers. Therefore we suggest that a study for any eco-geographic mechanism should also take small-scale factors into account, otherwise even if an eco-geographic trend is shown, it may result from microhabitat effects (for example, converse-Bergmann clines showed by Models 2 in Table 5.2 and Table 5.3).

From our results, it seems very likely that temperature is the mediating factor working on nest size and colony size through altitude and canopy cover. Two relationships support this hypothesis: between altitude, canopy cover and temperature, and between altitude, canopy cover and colony size (or nest size). First, altitude and canopy cover act on different elements of local thermal environments simultaneously: altitude on mean temperature and canopy cover on temperature fluctuation (Table 5.4, Fig. 5.3). Theoretically, temperature decreases with increasing altitude (Jacobson, 2005). We found an approximately 5$^{o}$C/km decrease in daily mean temperature along altitudinal gradients (Table 5.4). This is very close to the mean rate of 5.6$^{o}$C/km from a previous study in the Alps (Theurillat & Guisan, 2001) and the theoretical value of 6.5$^{o}$C/km (Jacobson, 2005). In terms of the canopy cover, thermal environments are colder and more stable in shadier areas than in open areas (Rodriguez-Garcia *et al.*, 2011; van Gils & Vanderwoude, 2012; Chen & Robinson, 2014). We found similar results for the thermal stability: temperature fluctuation was smaller in shadier areas than in open areas (Table 5.4, Figs 5.3b and 5.3d). To sum up, although altitude and canopy cover act on different elements of local temperature, increasing altitude and canopy cover have similar impacts: generally decreasing local temperatures (Fig. 5.5). Second, our results show that both altitude and canopy cover are associated with nest size and colony size (Table 5.2 and 5.3, Fig. 5.5). The negative interaction effects of altitude and canopy cover on nest size and colony size further indicate that the effects of altitude and canopy cover compensate each other in increasing nest size and colony size. A mediating factor shared by increasing altitude and canopy cover is one of the probable explanations. Accordingly, we suggest that temperature is the most likely mediating factor for the effects of altitude and canopy cover working on nest size and colony size.

Temperature has also been related to several hypotheses concerning the evolutionary-based reason of Bergmann’s rule. Fasting endurance was suggested to be the most likely hypothesis with respect to body size of endotherms: more seasonal environments favour larger body size because larger animals can store more fat and then survive during seasonal stress (Lindsey, 1966; Millar & Hickling, 1990; Blackburn *et al.*, 1999). Kaspari and Vargo (1995) propose a similar situation for colony size: large colonies can buffer the queen/s in resisting the harsh environment better than small colonies because of their greater energy reserves. We also consider thermoregulation as a candidate hypothesis. As mentioned before, our results indicate that canopy cover may have greater effects on nest size and colony size than altitude does. With lower daily maximum on local temperatures (Fig. 5.3b), thermal environments in shadier areas are cold for most of the time every day so they might not be able to provide enough heat for routine activities of a nest or a colony. With larger worker populations, a nest or colony would be able to produce sufficient endogenous heat generation, based on the metabolism and clustering behaviour of workers (Rosengren *et al.*, 1987; Frouz, 2000) and microbial heat production from nest materials (Coenen-Stass *et al.*, 1980; Frouz, 2000). Therefore, we hypothesise that having a larger nest size and colony size would be beneficial for thermoregulation.

Geraghty *et al.* (2007) studied Bergmann’s rule inter-specifically and found no significant relationship between altitude and colony size. This result may be due to the variation of colony size among species within the clades. A review study states that phylogeny has a greater influence on body size so inter-specific studies found

Bergmann’s cline less frequently than intra-specific studies did (Shelomi, 2012). Intra-specific study should therefore be most appropriate for studying eco-geographic rules. As far as we know, this is the first study aiming to apply Bergmann’s rule intra-specifically to the relationship between altitude and colony size of a social insect (Table 5.1). Although there are two species in our study, *F. lugubris* and *F. paralugubris* are two sibling species with similar functions in forest ecosystem, and have a sympatric distribution in the Swiss Alps (Bernasconi *et al.*, 2010). No species effect was revealed in any of our results (e.g. Table 5.2 and 5.3). We therefore consider this study as an effectively intra-specific study for the application of Bergmann’s rule to the relationship between altitude and colony size.

In terms of the colony-level organisation, our results show that monodomous colonies have larger nest size and smaller total colony size than polydomous colonies do (Models 6 in Table 5.2 and 5.3). Although a monodomous colony can build a larger individual nest, a polydomous colony can have larger total colony size by increasing nest number. We also find that there seems to be a trend that total colony size increases with increasing nest number per colony (Fig. 5.4). This further supports the results of a previous study which finds that colonies with more nests have larger total colony size (Chen & Robinson, 2014). In addition, colonies with more nests tend to have smaller variance in total colony size (Fig. 5.4). We do not find any effect of altitude nor canopy cover on the number of nests per colony; both these environmental factors may not affect colony-level organisation. We suggest that age could be a likely factor to explain the trends between total colony size and nest number per colony, and between the variance of total colony size and nest number per colony. Colonies with more nests may have been established for a longer time than those with fewer nests, therefore are larger with smaller variances than those of younger colonies. However, it would be challenging to test this hypothesis due to the lack of an effective method to measure the age of a colony. The definition of a polydomous colony we used may also affect the analyses for the colony-level organisation: more than 60% of colonies were defined as monodomous in this study (see sample sizes of colonies with different numbers of nests in Fig. 5.4). This functionally-based definition distinguishes polydomous colonies with one to more than twenty nests in the Peak District, UK (Chen & Robinson, 2014; Ellis *et al.*, 2014), and one to seven nests in this study in the Swiss Alps. The differences between studies may result from the differences between sampling sites.

To sum up, our study finds that nest size and colony size of *F. lugubris* and *F. paralugubris* follow Bergmann's rule along altitude when canopy cover is controlled for. To the best of our knowledge, this study is the first intra-specific study for the application of Bergmann's rule to the relationship between altitude and colony size. Second, the effect of canopy cover on nest size and colony size is more powerful than that of altitude. We therefore suggest that micro-climate or micro-habitat could be more important than the geographic factors in determining a life-history characteristic. Finally, temperature is the most likely mediating factor. Altitude and canopy cover may act on different elements of local temperatures simultaneously. Benefits of larger colony size (or nest size) on thermoregulation is the hypothesis we consider for the relationship between altitude, canopy cover and colony size. With regard to climate change, its effects may be vertically different along altitudinal gradients through multiple and complex ways (ie: changes in vegetation). Our study may provide a reference for future conservation actions regarding these red wood ant species which have ecologically significant impacts on the forest ecosystem.

# Acknowledgements

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# Appendices

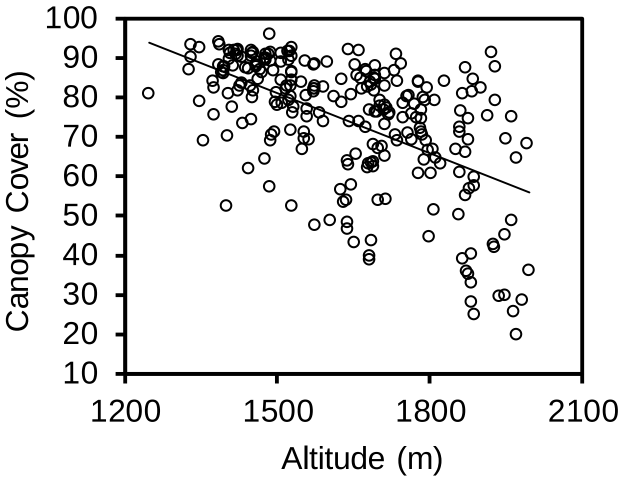

**Figure 5.S1.** Relationship between altitude and mean canopy cover of 253 colonies. Solid line shows the equation: Mean Canopy Cover (%) = 157.44 – 0.05 * Altitude (m). Data were fitted with linear regression ($F_{1,\,251} = 110.46$, $P < 0.001$, $r^2 = 0.31$).

**Table 5.S1.** The relationships between log$_{10}$ mean nest size, domy form, canopy cover and altitude. Data were fitted with linear model. The statistical baseline of “Species” factor was set as *Formica lugubris*. The statistical baseline of “Domy” factor was set as monodomy in Model 5 and 6. The “Interaction” term mean the interaction effect between canopy cover and altitude factors. The “anova” function was used for model selection. Model 5 was significantly better than Model 1 to 4 ($F = 7.75\text{-}36.89$, $P < 0.01$). There was no significant difference between Model 5 and 6 ($F = 0.68$, $P = 0.41$), the reduced model (Model 6) was therefore chosen as the best model.

| | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|
| | Estimate | *t* value | Estimate | *t* value | Estimate | *t* value |
| Intercept | 4.26e+00 | 28.58*** | 6.68e+00 | 19.68*** | 5.69e+00 | 103.69*** |
| Canopy Cover | 1.83e-02 | 9.42*** | - | - | - | - |
| Altitude | - | - | -6.48e-04 | -3.17** | - | - |
| Domy | - | - | - | - | -1.81e-01 | -2.43** |
| Species | 8.00e-02 | 0.22 | 7.21e-03 | 0.98 | 6.23e-02 | 0.83 |
| *F* | $F_{2,\ 250} = 45.14$*** | | $F_{2,\ 250} = 5.63$** | | $F_{2,\ 250} = 3.55$* | |
| $r^2$ | 0.26 | | 0.04 | | 0.03 | |

| | Model 4 | | Model 5 | | Model 6 | |
|---|---|---|---|---|---|---|
| | Estimate | *t* value | Estimate | *t* value | Estimate | *t* value |
| Intercept | -8.41e-01 | -0.54 | -7.49e-01 | -0.50 | -7.97e-01 | -0.53 |
| Canopy Cover | 7.58e-02 | 3.93*** | 7.47e-02 | 3.95*** | 7.57e-02 | 4.02*** |
| Altitude | 2.81e-03 | 3.27** | 2.79e-03 | 3.31** | 2.83e-03 | 3.36*** |
| Interaction | -3.14e-05 | -2.88** | -3.05e-05 | -2.85** | -3.11e-05 | -2.91** |
| Domy | - | - | -2.04e-01 | -3.27** | -2.10e-01 | -3.37*** |
| Species | 7.26e-02 | 1.14 | 5.19e-02 | 0.83 | - | - |
| *F* | $F_{4,\ 248} = 26.50$*** | | $F_{5,\ 247} = 24.17$*** | | $F_{4,\ 248} = 30.07$*** | |
| $r^2$ | 0.29 | | 0.31 | | 0.33 | |

**$P < 0.01$; ***$P < 0.001$

# Chapter 6 – General Discussion

Bergmann's rule may apply to the body size change across altitudinal gradients because of comparable changes of temperature between latitude and altitude. Just as body size is prominent to many biological aspects of an individual animal, colony size associates with many life-history features of social insects. Colony size therefore can be analogous to the body size of a social insect colony. In this thesis I investigated the relationship between altitude and ant colony size. The thesis contributes the first intra-specific evidence of Bergmann's rule applied to the colony size across altitude, and indicates not only the importance of microclimate on a fundamental characteristic of an animal but also the considerable role of microclimate for any study of eco-geographic trends.

In Chapter 2, I developed an accurate method to estimate red wood ant nest size, and indicated mound volume can be used as a feasible nest size index. Chapter 3 showed results of a preliminary test and indicated that microclimate may influence nest size. The case-study of Chapter 4 specifically investigated canopy cover effects on nest size and colony size of *Formica lugubris*. In Chapter 5, I finally conducted a study of the relationship between altitude, canopy cover, polydomy, nest size and colony size.

## Summary of Chapters

For studies regarding colony size and nest size, having a feasible and accurate estimation method is the fundamental and first step. Counting all individuals after nest excavation is definitely the most accurate method for assessing colony size (or nest size); however, the considerable drawback of this method is its destructiveness. Species of red wood ants are listed as “near threatened” by the IUCN (2014) and protected in many European countries (Bernasconi *et al.*, 2011). Complete excavation of nests is not considered as a routine method for studies which need to estimate wood ant colony size or nest size. In Chapter 2, I compared the accuracy and feasibility of four mark-release-recapture methods and a mound volume method with actual counts from nest excavation for estimating the nest size of *F. lugubris*. The results showed that gentle disturbance to the nest mound made the After-Disturbing method the most accurate mark-release-recapture method. There is a balance between accuracy, non-destructiveness, and time required for each method. Mound volume also can be a useful index of ant nest size with the lowest time requirement.

These methods were applied in a preliminary test for the relationship between altitude and colony size in Chapter 3. The monodomous population of *F. lugubris* in the Swiss Jura Mountains was targeted to simplify the question. This study did not show an altitudinal effect on colony size within a small geographic range. However, the study found that canopy cover and the aspect of nesting slope may be important for wood ant colony size. I therefore chose to use fisheye lens for the assessments of insolation aiming to include both canopy cover and the aspect of nesting slope. The results also led to the decision to carry out a further study in the Swiss Alps for a larger altitudinal range. Mound volume was then chosen to estimate nest size efficiently for obtaining the required sample size, which was calculated from power analyses.

Being aware of the importance of canopy cover and local temperatures of nesting location addressed in Chapter 3, I conducted an investigation focusing on the relationship between canopy cover, nest size and colony size in Chapter 4. An even-distribution sampling method was used to survey for colonies of *F. lugubris* in a part of the Peak District, UK (where *F. lugubris* is polydomous). The results indicated the association between larger nests and shadier areas, where the environments were colder and more stable compared to open areas. Colony size (sum of nest sizes in a polydomous colony) also tended to be larger in shadier areas than in open areas. I suggested that temperature and food resource availability may be the mediating factors between canopy cover and nest size.

Informed by the results from Chapter 2 to 4, I was able to investigate the relationship between a geographic gradient – altitude, an index of microclimate – canopy cover, polydomy, nest size and colony size in Chapter 5. This study was conducted on *F. lugubris* and *F. paralugubris* in the Swiss Alps with an altitude range of 1200 to 2100 metres. I found that total colony size and nest size of these two red wood ant species followed Bergmann's rule across altitudes (increase with increasing altitude) when canopy cover was controlled for. The results also showed that the effect of a small-scale factor (in this study, canopy cover) seemed to be more important than that of a geographic factor (altitude) in determining a life-history characteristic (nest size or colony size) of an animal.

Among these case studies, in Chapter 3, polydomous *F. paralugubris* had larger nest size than monodomous *F. lugubris* in the Swiss Jura Mountains (Fig. 3.2). I targeted monodomous nests in order to find *F. lugubris* colonies in this study, and thus inadvertently included some nests of *F. paralugubris* that appeared monodomous due to the lack of trails between nests during the surveys. Although I did not sample these *F. paralugubris* nests randomly, the role of domy form (polydomous or monodomous) in the differences of nest size in this study still interested me. In

Chapter 4, concerning polydomous *F. lugubris* in the UK, colonies with more nests had larger total colony size (Fig. 4.5) but tended to have smaller individual nests (Fig. 4.6). In Chapter 5, on *F. lugubris* and *F. paralugubris* in the Swiss Alps, monodomous colonies had larger nests than polydomous colonies (Table. 5.2, Fig. 5.1d). To set the results of these case studies in a broader context, in the Appendix section of this thesis, I report a systematic review conducted to investigate the relationship between domy form and nest size in red wood ants.

## Limitations and Future Work

Limitations of this thesis arose from the limited time frame, budget and labour force, and primarily concern two features of this thesis: limitations on the methods of nest size estimation and the observation-based nature of the studies.

### Methodological Limitations

Firstly, I mainly used mound volume method to estimate nest size and colony size of wood ants; mound volume of the nest was the actual parameter being tested. We cannot rule out the possibility that nests with similar worker population may build their nest mounds in different shapes under different environmental conditions. In Chapter 4 and 5, nests in colder environments (either in shadier areas or with higher altitudes) may construct higher mounds (Sudd *et al.*, 1977), thus resulting in larger mound volume. In addition, the mound volume method was also established on one population and at one time of one year (Chapter 2). However, in Chapter 3, mound volume indeed was also highly correlated to worker population (by After-Disturbing method) for both monodomous *F. lugubris* and polydomous *F. paralugubris* (Fig. 3.2). Therefore, mound volume can be used to estimate worker population of a nest in different populations. In the systematic review study, although the fitted relationships between mound volume and worker population from the two formulae (one from the Longshaw Estate in UK, the other from the Swiss Jura Mountains) are not the same, the difference between these formulae is in the intercept but not in the slope. This means that an increase in mound volume corresponds to a similar increase in worker population across different sites. With a limited time frame, mound volume was the only practical choice to obtain the required sample size, and it did function as an estimation of nest size with the best balance between effort, accuracy and time consumed. To clarify the effects of using mound volume, both mound volume method and the more accurate After-Disturbing method could be used simultaneously for future work, given a sufficient time frame and labour force. For example, comparing the nest size estimations from both methods can reveal whether nests with similar worker population may build higher mounds in shadier areas or at high altitudes to reach a smaller surface-to-volume ratio for better thermal conservation in order to cope with colder environments.

### Observation-Base Studies Limitations

The studies in this thesis are principally observational. In Chapter 4, although temperature was suggested as the main mediating factors between canopy cover and nest size, we still cannot completely separate the effects of temperature and food resource availability based on current observational data. However, the study is the first investigation specifically and systematically conducted for the relationship between canopy cover and nest size of wood ants. Given a longer time frame, budget and labour force, follow-up studies could include manipulation of canopy cover or food resource availability. For nests located in open areas, shelters could be constructed capping some nests, or extra food resources could be provided. For nests in shadier areas, heaters could be set up as extra heat sources, or some foraging trails could be blocked. Nest size estimation (worker population) could be performed every year or even every season on both experimental and control groups. The differences on nest size and colony size between these groups are very likely to be observed after several years because the development process of nest size might be slow.

Just as the Chapter 4 is the first study for canopy cover and wood ant nest size, the study in Chapter 5 also contributes the first intra-specific evidence of Bergmann's rule applying to the relationship between altitude and ant colony size. This investigation demonstrates the important role of microclimate for future geographic-scale studies. The manipulation of canopy cover and food resources described above could be performed across altitudes. Again, given a sufficient time frame, budget and labour force, follow-up studies could involve reciprocal transplants across altitudes or common garden experiments, where nests from various altitudes are transplanted in a controlled environment. With these manipulations, we could probably clarify whether the geographic difference is based on thermal plasticity or local adaptation. To conclude, although the case studies in this thesis are generally observation-based, this is an important first step to reveal ecological patterns. Subsequent studies can be designed specifically to identify probable underlying mechanisms.

### Other Future Work

Other aspects of future study could involve the application of Bergmann's rule to altitude and the body size of social insects. Heinze *et al.* (2003) showed a trend of larger body size in high altitudinal populations (Table 5.1). However the data in that study were only from two high-altitude populations which had considerably colder climate than expected from their latitude. Therefore, Bergmann's rule has not really been studied intra-specifically on body size along altitude in social insects. In Chapter 3, body size seemed to respond more sensitively to the change of a small altitude range than colony size did. Given that social insects have two levels of organisation, an investigation including both fundamental characteristics, body size and colony size, may reveal whether there is a trade-off or additive effect of these two levels of organisation on total biomass during the environmental change.

Future studies may also be able to broaden this question to all species in the red wood ant group. Studies in this thesis find intra-specific (or effectively intra-specific) patterns in two very closely-related red wood ant species. Although inter-specific studies are considered to be weaker than intra-specific studies in morphometric analyses (Shelomi, 2012), inter-specific studies would be required to reveal whether the patterns (colony size variations with altitude and canopy cover) we found are general, at least within a highly related group. Different species of red wood ants occur in different types of forests, for example, from open to deep or from young to old woodlands (Punttila, 1996; Kilpeläinen *et al.*, 2008; Punttila & Kilpeläinen, 2009), or from lowlands to mountains (Hågvar, 2005; Glaser, 2006; Bernadou *et al.*, 2015). However, some species have overlapping distribution. The species in the red wood ant group also have similar ecological roles. Inter-specific studies regarding canopy cover or/and altitude across the whole group may further reveal the differentiation of their ecological roles in the forest ecosystem.

# Conclusion

Climate change is probably the most momentous ecological and environmental issue of global concern. The impacts of climate change include alterations in species distribution and abundance, consequent effects on ecosystems and biodiversity (Parmesan, 1996; Hughes, 2000; Smith & Smith, 2003; Forero-Medina *et al.*, 2011). Rising temperatures is one of the main and general predictions of climate change (Smith & Smith, 2003). Temperature is the mediating factor which usually links climate change and altitude together. Climate change has different effects across this geographic feature (Diaz *et al.*, 2003; Beniston, 2006; Parmesan, 2006). For plants and many ectotherms, temperature has a profound impact on many functions relating to an organism's size, such as metabolic rates and rates of gas exchange (Atkinson, 1994). Therefore, climate change may affect animals through impact on body size mediated by rising temperature (Smith *et al.*, 1995; Hunt & Roy, 2006).

In this thesis, two accurate methods were firstly developed and demonstrated to be effective for estimating mound-building red wood ant nest/colony size, which can be considered the body size of a colony. Canopy cover effects were specifically investigated for their impacts on nest size and colony size of *F. lugubris*. Temperature was suggested to be one of the mediating factors between canopy cover and nest size. Bergmann's rule was then demonstrated to apply to total colony size and nest size of two red wood ant species across altitude, when canopy cover was controlled for. The small-scale factor (canopy cover) seemed to have a more important effect than a large-scale geographic factor did in determining a significant characteristic of an animal.

Montane species are greatly influenced by global climate changes which may result in impacts including population decrease, altitudinal range shift, and even species extinction (Pounds *et al.*, 1999; Walther *et al.*, 2002). The wood ants of the *Formica rufa* group are protected in many European countries due to their beneficial importance for woodlands (Bernasconi *et al.*, 2011). Therefore, understanding both large-scale geographic and local effects on the features and organisation of a wood ant colony has implications not only for predicting how they respond to environmental changes but also for understanding the broader effects of climate change on these ants and the forest ecosystem.

# Appendix – Polydomy and Nest Size

## Introduction

The individual and the colony are two levels of organisation for social insects. Some ant species have a nesting strategy named polydomy: a single colony can be spread into several spatially separated but socially connected nests (Debout *et al.*, 2007; Ellis & Robinson, 2014). The nest, an extra level of organisation for a polydomous colony, may also act as a unit of selection (Debout *et al.*, 2007). In the red wood ant group, gyny (multi-queen or single-queen) and domy (multi-nest or single-nest) are associated (Ellis & Robinson, 2014): monodomous colonies are usually monogynous, and polydomous colonies are always polygynous. Polygyny (one colony with multiple queens) is correlated with larger colony size in ants (Boulay *et al.*, 2014). A polydomous colony also can increase total colony size through increasing nest number to overcome the constraints on increasing size for a monodomous colony (one colony with only one nest) (Robinson, 2014). The question therefore arises: is there a trade-off between domy and the size of each nest? Or alternatively, can a polydomous colony increase total colony size by increasing nest number?

Interestingly, nesting strategy is flexible within species in the red wood ant group. For example, *F. aquilonia, F. lugubris* and *F. polyctena* have been recorded as polydomous in some areas but monodomous in others (Ellis & Robinson, 2014). This flexibility also provides an opportunity to investigate colony-level organisation intra-specifically. In this systematic review study, I therefore aimed to study the relationship between domy and nest size by investigating my data and of those from previous studies. In addition, populations in the same geographic region would be expected to be more closely related. Considering possible effects from population phylogeny, I therefore also included geographic region as a factor in the analysis.

## Materials and Methods

### Data Collection

To collect a dataset of domy and nest size for red wood ant species, I conducted a search of public databases (Web of Science, Google and Google Scholar) to scan the scientific literature. The name of each ant species (*F. lugubris*, *F. polyctena*, *F. aquilonia*, *F. rufa*, *F. pratensis* and *F. paralugubris*), the terms of domy (Monodomy or Polydomy), and the terms of nest size (Nest Size, Colony Size, Worker Population, Nest Volume and Mound Volume) were used as keywords. I included the data in my dataset if the literature contained all information of: sampling region, sample size, domy and nest size for a specific red wood ant species. Some data were complemented by personal communications from the authors (see Table 6.1 for more details on the procedure of literature search). Among red wood ants, *F. lugubris* is one of the most widely-distributed and well-studied species. Only the dataset of *F. lugubris* included sufficient data on both polydomous and monodomous populations for statistical analysis (Table 6.1).

**Table A.1.** Sources of nest size data on red wood ant group, their regions and the methods used in the studies.

| Species | Domy | Region | Method | n | Reference |
|---|---|---|---|---|---|
| *F. lugubris* | Polydomous | British Isles | M-V | 364 | Sudd *et al.* (1977) |
| | | British Isles | M-V | 197 | Chen and Robinson (2014) |
| | | British Isles | M-V (Real Counting) | 16 (11) | Chen and Robinson (2013) [a] |
| | | British Isles | M-V | 55 | Borkin *et al.* (2012) [b] |
| | | Mainland Europe | M-V | 286 | Y.-H. Chen, Unpublished data (Chapter 5) |
| | | North America | M-V | 93 | Storer *et al.* (2008) [c] |
| | Monodomous | Fennoscandia | M-V | 1 | Kilpeläinen *et al.* (2008) |
| | | Mainland Europe | M-V | 26 | Y.-H. Chen, Unpublished data (Chapter 3) |
| | | Fennoscandia | M-V | 58 | Punttila and Kilpeläinen (2009) [d] |
| | | British Isles | MRR | 5 | Breen (1979) |
| *F. polyctena* | Polydomous | Fennoscandia | M-V | 7 | Kilpeläinen *et al.* (2008) |
| | | Fennoscandia | M-V | 12 | Härkönen and Sorvari (2014) [b] |
| | | Fennoscandia | M-V | 12 | Punttila and Kilpeläinen (2009) [e] |
| | Monodomous | Fennoscandia | MRR forager, M-V | 1 | Rosengren (1977) |
| *F. aquilonia* | Polydomous | Fennoscandia | M-V | 60 | Laakso and Setälä (1997) |
| | | Fennoscandia | M-V | 358 | Kilpeläinen *et al.* (2008) |
| | | British Isles | M-V | 89 | Borkin *et al.* (2012) |
| | | Fennoscandia | M-V | 302 | Punttila and Kilpeläinen (2009) [e] |
| *F. rufa* | Monodomous | Fennoscandia | M-V | 4 | Kilpeläinen *et al.* (2008) |
| | | Fennoscandia | M-V | 15 | Punttila and Kilpeläinen (2009) [d] |
| *F. pratensis* | Monodomous | Fennoscandia | M-V | 2 | Punttila and Kilpeläinen (2009) [d] |

n = number of nests in the literature; M-V: measurement of mound volume (usually as semi-ellipsoid) or dimensions of mound (two diameters and height); MRR: mark-release-recapture method; [a] with both real counting and mound volume data (see Chapter 2), considering random sampling, the latter was used; [b] complemented by personal communications from the authors; [c] with conical volume (converted to semi-ellipsoid); [d] only mentioned as monogynous (very likely to be a monodomous population); [e] only mentioned as polygynous (very likely to be a polydomous population).

## Nest Size Conversion

Most published studies contained only the nest mound dimensions (either volume or diameters and height; Table 6.1) because worker population counts are difficult in red wood ants. According to previous study (Chapter 2: Chen & Robinson, 2013) and unpublished data (Chapter 3: preliminary test, see Table 3.2), worker population of a red wood ant nest is highly associated with its mound volume. I therefore used two alternative formulae in order to convert mound dimension data into estimated worker population. The first formula was based on the data on *F. lugubris* and *F. paralugubris* collected in the Swiss Jura Mountains in 2012 (Chapter 3). The second formula was based on the data on *F. lugubris* collected in the Longshaw Estate, UK in 2012 (S. Ellis, personal communication). In both formulae, nest size (worker population) was estimated by After-Disturbing method (a mark-release-recapture method; Chen & Robinson, 2013) and mound volume was calculated as a semi-ellipsoid by two diameters and height. Both nest size and mound volume values were transformed by $\text{Log}_{10}$ to normalise the distributions. Linear regression models were used for the relationship between worker population and mound volume.

Formula 1: $\text{Log}_{10}$ Worker Population = 0.6374 + 0.6435* $\text{Log}_{10}$ Mound Volume ($\text{cm}^3$)

($F = 129.15$, $P < 0.001$, $r^2 = 0.77$, n = 41)

Formula 2: $\text{Log}_{10}$ Worker Population = 1.7634 + 0.4980* $\text{Log}_{10}$ Mound Volume ($\text{cm}^3$)

($F = 6.62$, $P < 0.05$, $r^2 = 0.42$, n = 11)

These two formulae differ in the intercept (regression model, $t = 3.34$, $P < 0.01$, n = 52). However, there is no significant difference in the slopes ($t = 0.94$, $P = 0.35$) of two formulae. Although Formula 1 was more precise (better fitted model with higher $r^2$ value) than Formula 2, it did not mean that the former can better predict the accurate value of actual worker population. In order to compare the accuracy of these Formulae, I used the data from our previous study (Chapter 2: Chen & Robinson, 2013) which contained both mound volume and real worker population counts. The two Formulae were used to generate estimated values of worker population from the mound volume data in Chapter 2, then the estimated worker population values were compared with the real counting of worker population. Although Formula 2 had lower r-square value than Formula 1 did, the estimated worker population converted by Formula 2 was closer to the real counting value of worker population. I thus chose Formula 2 to convert nest size data for the following analyses.

### Statistical Analyses

If both worker population data and mound volume data were present in the literature, the former were used for the analysis of nest size. All nest size data were $\text{Log}_{10}$ transformed for further analyses. Populations from continental Europe were separated into Fennoscandia and Mainland Europe groups. Overall, regions of the populations which the data were collected from were divided into four groups: North America, the British Isles, Fennoscandia and Mainland Europe. I used two-way ANOVA to test whether the region and the domy of the population have effects on the mean nest size of *F. lugubris*. Sample size of each population (how many nests contribute to the nest size value in the literature) was used to give different weights to each population-level data point. Tukey's HSD was used for post-hoc comparisons. Statistical tests were performed using JMP package (version 6.0.0; SAS institute, Cary, NC, USA).

## Results

I obtained nest size data on six populations of polydomous and four of monodomous *F. lugubris* from published literature, personal communications with authors and unpublished data (Table 6.1, Fig. 6.1). Polydomous and monodomous populations of *F. lugubris* did not significantly differ in nest size ($F_{1,\,9} = 0.32$, $P = 0.60$; overall ANOVA model: $F_{4,\,9} = 7.99$, $P < 0.05$, sum weights = 1101). Populations of *F. lugubris* in different regions had significantly different mean nest size ($F_{3,\,9} = 10.45$, $P < 0.05$, Fig. 6.2): nest sizes of both populations in North America and Mainland Europe were significantly larger than that of the populations in the British Isles; populations in North America, Mainland Europe and Fennoscandia did not significantly vary in nest size; nest sizes of populations in Fennoscandia and the British Isles were not significantly different.

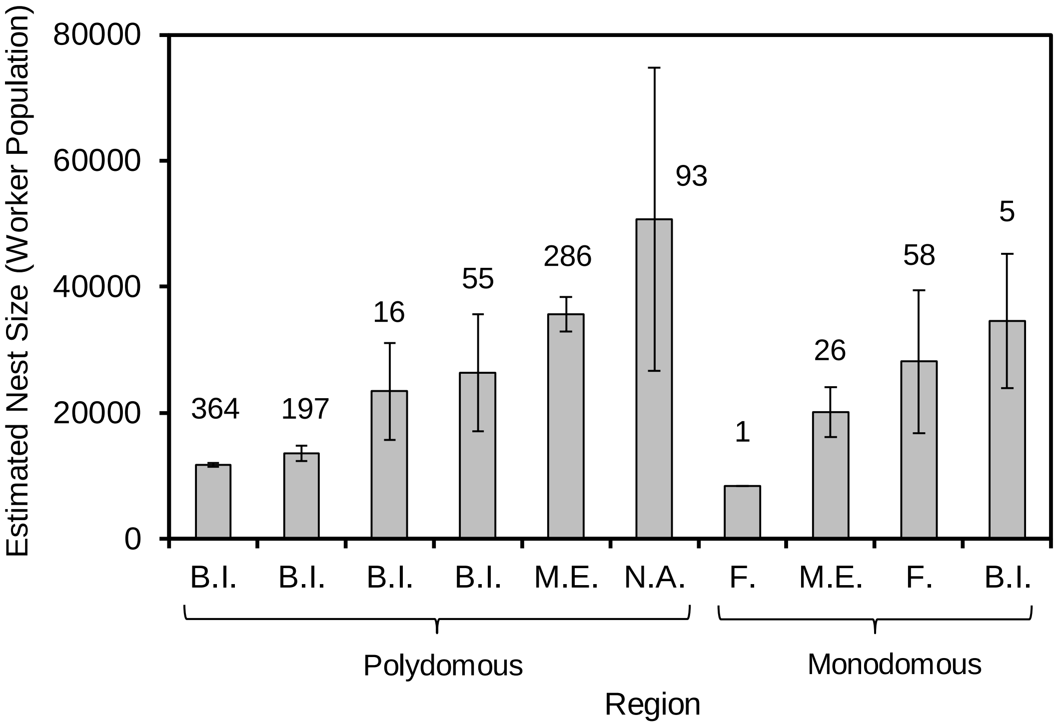

**Figure A.1.** Estimated nest size (worker population) of *Formica lugubris* of each population. The number above each bar indicates sample size of each study. Error bar shows 1SE; B.I.: the British Isles; M.E.: Mainland Europe; N.A.: North America; F.: Fennoscandia.

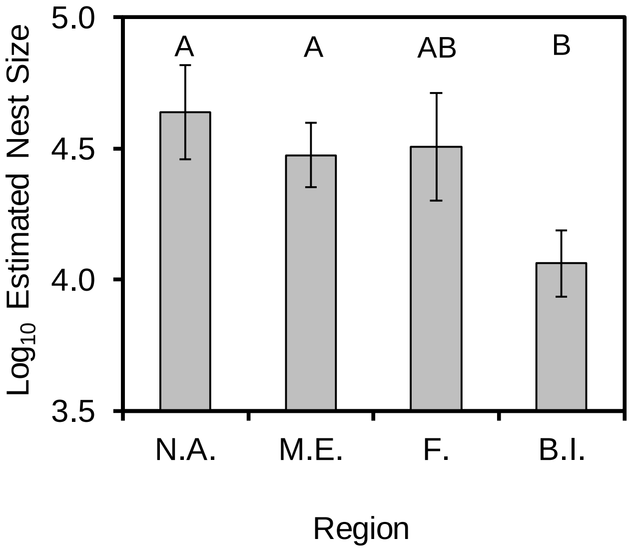

**Figure A.2.** $Log_{10}$ estimated nest size (worker population) of *Formica lugubris* in various geographical regions. Different letters indicate significant difference among groups. Error bar shows 1SE; B.I.: the British Isles; M.E.: Mainland Europe; N.A.: North America; F.: Fennoscandia.

## Discussion

If there was a trade-off between nest size and nest number, we would expect that polydomous populations had smaller nest size. However, I find that monodomous and polydomous populations did not differ in nest size. Therefore there is no evidence for a trade-off between nest size and nest number. The finding from this systematic inter-population review contrasts with our previous results of intra-population studies, which show that polydomous colonies with more nests tended to have smaller nests (Fig. 4.6) and polydomous colonies had smaller nests than monodomous colonies did (Table 5.2, Fig. 5.1d). The "domy form" therefore may not have a universal effect across populations, or there may be other factors which play more important roles than domy form in determining nest size at the population level.

Instead of the domy effect, nest size showed a geographical inter-population variation. Three reasons could cause these nest size differences found in this study. The first reason arose with the methods used for estimating nest size. I applied a single formula for all populations to convert mound volume into worker population data. It is possible that this formula was only accurate to convert those data for the populations of the British Isles because the formula was derived from a study in England. Although I converted the data to an estimate of worker population, in the main I was actually in effect comparing mound size between populations (only one study had worker population data). Our two formulae, which were used to convert mound volume to worker population, only differ in the intercept but not in the slopes. This means that nests with similar worker population from two different regions (populations) may build different mound sizes. However, the results also mean that mound volume predicts similar increasing trends of worker population with increasing mound volume in two different populations. Using population-specific formulae which are based on each population would be expected to provide more accurate nest size values. However, I did not have both mound volume and worker population data of the populations in Fennoscandia and North Europe, so applying one formula consistently was the best remaining option to avoid bias.

Secondly, the variation in nest size across populations may be associated with the phylogeny of these populations. From a phylogenetic perspective, populations in the British Isles are closer to the populations in Fennoscandia than to the populations in other regions (Pamilo *et al.*, 1992). The results of this study also match this phylogenetic pattern: there was no difference between the populations in the British Isles and Fennoscandia in nest size. In addition, the *F. lugubris* population in North America was transported for introduction from Italy (Mainland Europe) into Quebec, Canada in the 1970s (Finnegan, 1975). There was also no difference between the populations in Mainland Europe and North America groups in nest size. It is very likely that nest size, a fundamental characteristic of ant colonies, has some genetic basis among these populations.

Finally, as demonstrated in Chapters 4 and 5, both geographic factors and local microclimate impact wood ant nest size. On one hand, these populations are distributed across a large geographic scale (latitude: 46.3-63.1$^{o}$N, altitude: 200-2000 metres). The climate and ecological conditions vary in the regions of these populations. However, the geographic information from literature was not sufficient for further analysis of these effects. On the other hand, local habitat is another considerable factor. Taking the populations of our previous studies as examples; the conditions of habitats differ between the British Isles (Scotland and England) and Mainland Europe (the Swiss Jura Mountains and the Swiss Alps). According to our observation, woodlands are more fragmented in Scotland and England than in Switzerland. Fragmentation results in more edge areas of forests. Canopy cover data also support our observation: nests in the Swiss Alps were located in areas with higher canopy cover (Canopy cover = 75 ± 16%, Mean ± 1SD, Chapter 5, n = 426) than those in England (Canopy cover = 59 ± 15%, Mean ± 1SD, Chapter 4, n = 201) (Kruskal-Wallis test: $\chi^2 = 135.49$, $P < 0.001$). In Chapter 4, we found that nests located in shadier areas were larger than those in open areas. The results of this review study conform to our empirically observed relationship between canopy cover and nest size: nests of the populations in the British Isles were smaller and located with lower canopy cover, whereas those in the Swiss Alps were larger with higher canopy cover.

Briefly summing up, we suggest three reasons which could cause the geographical inter-population variation of nest size: firstly, I applied a single formula (from one of the populations in several regions) for all populations to convert mound volume into estimated worker population; secondly, the variation in nest size across populations in different regions is very likely associated with the phylogeny of these populations; finally, the variation in nest size across populations may be linked to the differences of large-scale and/or small-scale environmental factors of different regions. However, with the data I collect from both my studies and literature, it is not possible to achieve any firm conclusion; more data would be needed. A global database for the biodiversity of ants has been curated by Dunn *et al.* (2007). Ant studies that focused on canopy cover are included in the database. To include some basic features of ant colony (e.g. colony size) would be useful for future studies on the relationships between environmental factors and characteristics of ant populations.

In addition to the effect of domy form on nest size among populations, the investigation presented in Chapter 5 includes an intra-population relationship between domy form and nest size. For *F. lugubris* in the Swiss Alps, I defined it as a polydomous population in this systematic review according to two factors. Firstly, more than two thirds of the nests were polydomous (193 of 286 nests). Secondly, we used the functional definition of polydomy in which the nests are defined as the same colony if they are linked together by trails (Heller & Gordon, 2006; Gordon & Heller, 2012). The functional definition is stricter than the other commonly used definition in which the individuals from the same colony have non-aggressive communication (Pedersen & Boomsma, 1999). In the Swiss Alps, those nests without any trail linked to other nests were defined as monodomous colonies by the functional definition. According to the aggressiveness definition, if those functionally-defined monodomous nests were not aggressive to the nearby one/s, they may belong to the nearby nest/s to form a polydomous colony.

This systematic review study shows that domy form did not have effect on nest size of *F. lugubris* across populations. This result contrasts with the finding of a previous intra-population study which shows that monodomous colonies had larger nest size than polydomous colonies did (Chapter 5). Instead, this review study shows that the populations in different geographic regions differed in nest size. The variation pattern in nest size mostly conforms to the phylogeny between these populations. The effects of environmental factors (in both geographic and local scales) may also be the reasons for the inter-population differences in nest size.

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