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Combined effects of temperature and interspecific competition on the mortality of the invasive garden ant, *Lasius neglectus*: A laboratory study

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ABSTRACT

The invasive garden ant, *Lasius neglectus*, is a dominant species due to its capacity to form large supercolonies. This species was assumed to possess a wide thermal niche since it is able to adapt to cold climates, which is a factor that boosted its rapid expansion from south to many central-northern European Countries. However, the effect of variations in environmental temperatures on its competitive ability against other species has still not been investigated. In this paper, we analyzed the change in survival ability of *Lasius neglectus* during encounters with two Mediterranean dominant ants (*Crematogaster scutellaris* and *Tapinoma nigerrimum*) at four different temperatures (15, 20, 25 and 30 °C). Firstly, control tests were performed to provide the baseline survival ability of the three species at different temperatures. Secondly, competition tests were carried out at the same temperatures. *Lasius neglectus* survival was negatively affected by high temperature (30 °C) in control tests, and this impairment was much more pronounced in competition tests. On the contrary, the two opponent species were only marginally affected by temperatures in control tests. *Crematogaster scutellaris* was a better competitor than *L. neglectus*, particularly at high temperatures. *Tapinoma nigerrimum* was a weaker competitor and was always outcompeted by *L. neglectus*, particularly at low temperatures. This result could suggest that *L. neglectus* is at a disadvantage during interspecific encounters when temperatures are high and that the predicted future increase in environmental temperatures may potentially enhance this handicap.

1. Introduction

The spatial distribution of all organisms is influenced by the complex combination of biogeographical factors, the ability to tolerate specific abiotic conditions (including those induced by human actions), and interactions with other organisms (Wallis, 1962; Ruby et al., 1994; Chen and Nonacs, 2000; Canoine and Gwinner, 2002; Grover et al., 2007; Huffard et al., 2010; Ottonetti et al., 2010). The ability of a species to successfully cope with other organisms (e.g. compete) may be considerably different when external abiotic conditions (such as temperature or water availability) are optimal, sub-optimal, or adverse. For example, it is known that the chances of success in a fight may be far higher when the environmental temperature or air humidity are within optimal limits than when these conditions are not fully suitable (e.g. Zamudio et al., 1995; Briffa and Elwood, 2000; Arnott and Elwood, 2009).

The importance of temperature as an ecological factor structuring ant communities is well documented (e.g. Cerdá et al., 1998a; Bestelmeyer, 2000; Sanders et al., 2007; Reymond et al., 2013). In general, ants are considered thermophilic organisms, although the thermal tolerance of different species is variable (Diamond et al., 2012). Daily changes in environmental temperature regulates the activity patterns of the species within a community (Retana and Cerdá, 2000; Santini et al., 2007, Arnan et al., 2012,) reducing the overlap of dominants and subordinates and contributing to enhancing species coexistence (e.g. Cerda et al., 1997; Stringer et al., 2007; Lessard et al., 2009).

The irruption of invasive alien species can unbalance these systems and may disrupt competition hierarchies. For example in northern California, the invasive Argentine ant *Linepithema humile* (Mayr, 1868) outcompeted several autochthonous species due to the wider thermal niche that allows this species to have longer daily activity periods compared to many of the autochthonous species (Human and Gordon, 1996). Additionally, changes in temperature and water availability due to climate change may further unbalance ecological systems, particularly when these changes have different effects on the species (Walther et al., 2002; Menke and Holway, 2006; Parmesan, 2006). The comprehension of ecological dynamics involving alien and invasive species according to thermal conditions can, therefore, be of the utmost importance to understanding the future arrangement of biological

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communities due to anthropogenic climate change (Fagan et al., 2014; Bertelsmeier et al., 2015b). Trying to predict the potential distribution of alien species and their ability to spread over unexploited areas is, indeed, a key challenge for biodiversity conservation (Vitousek et al., 1997; Dukes and Mooney, 1999; Occhipinti-Ambrogi, 2007; Bertelsmeier et al., 2015b).

The invasive garden ant, *Lasius neglectus* van Loon et al., 1990 (Hymenoptera: Formicidae), is a species described for the first time in 1990 (van Loon et al., 1990), although it probably started to spread in Europe a couple of decades previously due to accidental transfer (Seifert, 2000). Today, this species has become established throughout the majority of Europe causing documented environmental and economic damages (Seifert, 2000; Tartally et al., 2004; Espadaler et al., 2007; Ugelvig et al., 2008; Nagy et al., 2009), although a recent account of the status of its populations demonstrated a decrease in invasiveness (Tartally et al., 2016). This species is known to form vast supercolonies comprising hundreds of million workers and several thousand queens, and it is a dominant competitor due to the huge number of individuals which can be involved both in food exploitation and in fights against other species (Espadaler et al., 2007; Bertelsmeier et al., 2015a, 2016). Additionally, *L. neglectus* is reputed to possess a wide thermal niche given its rapid expansion both in Southern and in Northern European countries (Ugelvig et al., 2008). In particular, the assessed presence of this species in areas characterized by severe winters (with mean temperatures around −5 °C) suggest that *L. neglectus* could easily adapt to cold climates (see Seifert, 2000), where other species have a disadvantage. Pamminger et al. (2016) recently demonstrated that increasing temperature negatively affects the immune system of this species, reducing its resistance to *Beauveria bassiana* (Bals. - Criv.) Vuill. 1912 (Ascomycota: Sordariomycetes) infections. This factor may limit the spread of the species in warmer areas. However, the effect of variations in environmental temperatures on the competitive ability of this species towards autochthonous species has not been investigated yet. In this laboratory study, we first assessed the short-time baseline survival of *Lasius neglectus* and two autochthonous species that are found widespread along the Mediterranean basin, *Crematogaster scutellaris* (Olivier, 1792) and *Tapinoma nigerrimum* (Nylander, 1856) at different temperatures. Secondly, we investigated how the survival ability of *Lasius neglectus* is affected by temperature changes during inter-specific encounters with the same two species. We chose these two opponents due to their dominant role within Mediterranean ant communities. Both usually form large colonies, actively defend resources, and can outcompete other species (Retana and Cerda, 1994; Cerda et al., 1998b; Marlier et al., 2004; Santini et al., 2011, Frizzi et al., 2015). Due to their ecological role, these two species could be considered possible obstacles to the spread of *L. neglectus* in the Mediterranean area. Finally, both these species are known to be adapted to warm habitats and are therefore expected to be better competitors at higher rather than lower temperatures. The genus *Crematogaster* is mainly a tropical/subtropical genus, and *C. stutellaris* can maintain active colonies even during the hottest times of the day (Santini et al., 2007). Similarly, *T. nigerrimum* is widespread in the warmer areas of southern Europe (Seifert, 2012).

2. Materials and methods

The study was carried out from June to September 2012. The ants were collected from sites within a distance less than 20 km from Florence (Italy). The climate of the area is typically Mediterranean with mild cold winters and hot, dry summers. *Lasius neglectus* were collected from two separate areas: the public gardens in the city of Prato (Italy) and along the riverside of the Greve River (Scandicci, Florence, Italy). *Tapinoma nigerrimum* and *Crematogaster scutellaris* workers were collected at the University Science Campus in Sesto Fiorentino (Florence, Italy). For each species, ants from at least ten different nests, located at least 50 m apart, were collected, and their test order was randomized to avoid nest-related effects as much as possible. However, *L. neglectus* forms large supercolonies with no evident distinction between different nests (Cremer et al., 2008), and it was, thus, impossible to be confident that the selected nests within each area were independent. Average worker size (linear length from mandibles to the VII abdominal tergite) was 2.72, 3.25, and 4.56 mm for *Lasius neglectus*, *Tapinoma nigerrimum,* and *Crematogaster scutellaris*, respectively.

Workers were collected during the morning (8:00–10:00 am) and left to acclimatize to laboratory conditions with water available for 24 h under natural illumination before being used in the experiments to reduce manipulation stress and avoid using ants accidentally injured during collection. During acclimation to laboratory conditions ants were exposed to a temperature ranging from 22 and 27 °C, a range similar to the one naturally experienced in the field. Groups of 10 ants were maintained in a 50 ml plastic vial to rule out any effect of group size on the aggression tests (Tanner, 2006), adding a moistened cotton flock to provide 100% relative humidity and avoid dehydration. After acclimation, the tubes were placed within a thermostatic chamber for 60 min and gradually changed to one of the chosen test temperatures (15, 20, 25 and 30 °C), before starting the experiments. The selected temperatures were comparable to the average of the maximum values occurring during the spring and summer, i.e. the main activity periods for ants in the Mediterranean area (Cros et al., 1997; Santini et al., 2007). Tests were performed within a neutral arena (9 cm diameter Petri dish) with Fluon© coated walls to prevent workers from escaping. During all the tests, a cotton flock soaked with water was placed in the center of the experimental arenas to prevent dehydration, and the relative air humidity was maintained constantly at 70%.

Two different types of tests were performed. The first test, hereafter referred to as “controls”, aimed to quantify the baseline species' survival at the four temperatures. In this case, groups of 10 conspecifics (sampled from the same nest) were placed in a test arena at the experimental temperature. In this case there was not any intraspecific aggression, given that all ants in a sample come from the same nest. For the second type of experiment, hereafter referred to as “competition tests”, ten *L. neglectus* and ten workers of one of the opponent species were simultaneously dropped into the arena and exposed to one of the experimental temperatures. Each day, an equal number of all the possible test combinations were carried out to minimize errors due to experimental conditions: *Lasius-Crematogaster* and *Lasius-Tapinoma* competition tests, and the same number of control tests for each of the three species. For each test type and temperature combination, 20 independent replicates were obtained giving a total of 240 control tests (3 species x 4 temperatures x 20 replicates for each combination of species and temperature) and 160 competition tests (2 types of encounters x 4 temperatures x 20 replicates). Each arena was observed every 60 min for six hours, and the number of surviving workers per species was recorded. Despite aggressive behaviors and prolonged fights were observed when checking the number of survivors during competition experiments, no explicit recording of ant behavior was carried out, and hence we use survival as a proxy for competitive ability. The experimental thermostatic chamber had a double layer door; the inner door is made of glass and is transparent, which allowed observations to be recorded without altering the internal temperature.

The time series of the number of dead and surviving ants were fitted using the Kaplan-Meier estimator curves (Bland and Altman, 1998) for survival data. Survival during both control and competition tests was modeled using Cox proportional hazards regression models with Efron approximation to handle time ties (Andersen and Gill, 1982; Hertz-Picciotto and Rockhill, 1997). Complete models included temperature as the main factor, and a random frailty term to account for non-independence of individuals within each batch of ants (Therneau et al., 2003). Statistical significance of the fitted models was assessed using a likelihood ratio test, comparing the full model with a null model