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Where to move when it gets cold: winter nesting sites attractive to the Argentine ant (Hymenoptera: Formicidae)

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Abstract

The Argentine ant, *Linepithema humile* (MAYR, 1868), shows a cycle of regrouping of its nests in winter. Nests are located in similar sites every year, with a high density of queens in winter. We measured the physical characteristics, temperature and water content of 90 winter nests and control points, from December 2008 to March 2010. Additionally, we carried out a bimonthly monitoring of nest site fidelity throughout 2009 and in winter of 2009 / 2010. All studies were conducted in two invaded areas of the north-eastern Iberian Peninsula. The objective was to determine the preferred places for this species to build its nests in winter. Our results showed that nest location was influenced primarily by soil moisture, temperature and the vegetation around. Soil moisture inside nests was regulated mainly by temperature which in turn was controlled by orientation, rock color and canopy cover above the nest site. Canopy cover was related to the distance at which the nests were from plant structures, providing them a close food resource. Additionally, canopy cover could also play a two-side role helping to avoid high levels of soil moisture and extreme temperatures inside nests. Nest orientation and rock color may also help to protect the colony from extreme temperatures as well as to capture sunlight to provide an additional heat source maintaining optimal environmental conditions inside the nest, but also for colony activity during winter. Knowledge of the Argentine ant's nesting behavior is essential to initiate control methods such as the extirpation of winter nests with the consequent elimination of high densities of queens and their brood in invaded natural areas.

Key words: Winter nest, nest site fidelity, canopy cover, nearest tree, soil water content, winter nest temperature.

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Introduction

The Argentine ant, *Linepithema humile* (MAYR, 1868), is a worldwide invasive species introduced into Mediterranean-type climate areas as a result of commercial activity (HÖLLDOBLER & WILSON 1990, PASSERA 1994, SUAREZ & al. 1998, 2001, ROURA-PASCUAL & al. 2011). Its aggressive behavior (CARPINTERO & REYES-LÓPEZ 2008, ABRIL & GÓMEZ 2011) and its numerical dominance have a negative impact on native ant species, arthropod communities (HUMAN & GORDON 1996, HOLWAY 1998, SUAREZ & al. 1998, OLIVERAS & al. 2005), ant-vertebrate interactions (SUAREZ & al. 2000, ESTANY-TIGERSTRÖM & al. 2010), and ant-plant relationships (BOND & SLINGSBY 1984, VISSER & al. 1996, GÓMEZ & OLIVERAS 2003, GÓMEZ & al. 2003, BLANCAFORT & GÓMEZ 2005, RODRÍGUEZ-CABAL & al. 2009), thereby causing a drastic decrease in the biodiversity of the invaded areas and altering important ecological processes (DEL TORO & al. 2012).

The Argentine ant is heavily influenced by temperature and water availability. These two factors, along with others (e.g., vegetation around, available food sources), are the key to its establishment in new areas (ROURA-PASCUAL & al. 2004, 2006, JUMBAM & al. 2008). Temperature has a strong influence on some of the species' reproductive traits, such as the queen's oviposition rate (BENOIS 1973, ABRIL & al. 2008a), brood development rate (NEWELL & BARBER 1913, BENOIS 1973, ABRIL & al. 2008a), and foraging effectiveness (MARKIN 1970, HUMAN & GORDON 1999, WITT & GILIOMEE 1999, HOLWAY & al. 2002, ABRIL & al. 2007, JUMBAM & al. 2008). Water availability determines the abundance and distribution of the Argentine ant in Mediterranean-type systems (HUMAN & GORDON 1999, SUAREZ & al. 2001, HOLWAY & al. 2002, JUMBAM & al. 2008) and plays an important role in its colony survival. It has been reported that the Argentine ant has significantly higher rates of water loss and cuticular water permeability than native ant species adapted to dry and hot Mediterranean environments (SCHILMAN & al. 2007). This is likely the reason why in low humidity environments its foraging activity is negatively affected by the influence of high air temperatures (HUMAN & GORDON 1999, HOLWAY & al. 2002, ABRIL & al. 2007) and why low soil moisture limits its expansion (HOLWAY & al. 2002, MENKE & HOLWAY 2006, MENKE & al. 2007). In invaded natural areas, the Argentine ant changes its nesting preferences seasonally according to food availability (HELLER & al. 2006) and its physiological temperature and humidity requirements (NEWELL & BARBER 1913, MARKIN 1970, BENOIS 1973, HELLER & GORDON 2006, ABRIL & al. 2008b). In winter, the spatial range of the colony contracts into large aggregations called "winter nests" (NEWELL & BARBER 1913), characterized by a large number of workers and queens (ABRIL & al. 2008b). Ants usually show preferences for nesting sites (FERNÁNDEZ-ESCUDERO & al. 1993) and in temperate zones they build their nests mostly under rocks (HÖLLDOBLER & WILSON 1990) which protect them from predators and extreme temperatures, providing them with optimal thermal regimes (FERNÁNDEZ-ESCUDERO & TINAUT 1999, TINAUT & al. 1999, THOMAS 2002, ROBINSON 2008, MCCAFFREY & GALEN 2011). The Argentine ant is also known to build its nests generally under rocks (COLE & al. 1992, INGRAM 2002) and in a very similar location in winter year after year (HELLER & GORDON 2006), which means that this species shows a certain fidelity to the areas where winter nests are located. The nests are usually built in the top 35 cm of the soil and are of a basic structure (NEWELL & BARBER 1913, MARKIN 1970, HELLER 2004, HELLER & GORDON 2006). As a consequence of being so shallow, abiotic factors inside the nest can be highly variable. The seasonal location of nests therefore depends on several physical and environmental factors that may influence temperature and humidity conditions, and hence determine the most suitable areas to nest.

Attempts to eradicate established populations of Argentine ants in invaded natural areas have had little success. The use of toxic baits has reduced worker populations but seems to have failed to exterminate the queens, which control the reproductive potential of the colony (KRUSHELNYCKY & REIMER 1998a, 1998b). In areas where the invasion is well-established the most effective way of control is to slow its rate of spread, and limit its establishment in other non-invaded areas. As the use of wide-spectrum chemicals is not allowed in some areas of natural interest, one possible method of control could be the removal of queens during winter, which is the period of maximum queen densities inside nests (ABRIL & al. 2008b). This method could be suitable for slowing the invasion rate on a local scale or in small and recently invaded areas. With this in mind, the correct location of Argentine ant winter nests is an important key (TSCHINKEL 2011) for an effective management of the invasion, based on the extraction and elimination of queens in the advancing front. The aim of the present study was therefore to determine the nesting preferences of this invasive species in winter, in order to locate winter nesting places easily.

Material and Methods

Study area: The study was conducted in two invaded localities of open cork oak secondary forests on the southern edge of the Gavarres Massif, in the areas of Santa Cristina d'Aro (CA, 41° 48' 51.71" N, 3° 01' 50.57" E) and Pedralta (PD, 41° 47' 31.53" N, 2° 58' 52.79" E), in the northeast (NE) of the Iberian Peninsula. This region has a Mediterranean climate with 690 mm of mean annual rainfall and a mean temperature of 15.5°C (Database of Automatic Meteorological Stations (EMA) of the Catalan Government).

Argentine ant nest monitoring and the measurement of environmental characteristics of winter nests were carried out from mid-December 2008 to mid-March 2010. Nest site fidelity was monitored every two months throughout 2009 and again in winter 2009 / 2010. In winter 2009 / 2010 control points were included in the study to compare the effects of physical and environmental factors inside and outside the winter nests.

Physical factors: To determine the physical characteristics of winter nests we randomly chose a total of 90 nests (50 in CA and 40 in PD forests), and registered the following variables for each of them: canopy cover above the nest (%), orientation (i.e., the main direction towards which the nest rock was facing taking into account the direction of the nest slope), distance to the nearest nest (m), to the nearest tree (m), and to the nearest human-made path (i.e., walking tracks, (m)). We recorded the location of each nest using a Garmin eTrex Legend® HC × GPS with an accuracy of three meters, as well as orientation and distance to the nearest nest (calculating the Euclidean distances with Mapsource_v6.13.7 Extreme GPS software). When the nest was located under a rock, we also measured its surface in cm^2 (maximum × minimum diameter), and its color. The color of the rock was categorized as follows: (1) light colored and (2) dark colored or colonized by lichens or mosses, taking a color standard as shown in Figure 1. Finally, we measured the canopy cover with digital photographs of the coverage at each site. We analyzed the pictures with GapLightAnalyzer_v2 software, which estimates the percentage of canopy openness. The canopy cover (%) was calculated then as: 100 - canopy openness (%).

We also sampled 90 control points free of Argentine ant nests, located in a random direction 2 m away from each sampled nest. Canopy cover was also measured for the control points using the same method as for winter nests.

Temperature and water content: Throughout the two winters, we measured the soil temperature (°C) and soil volumetric water content (VWC, %) of the 90 winter nests. In the second winter we also assessed these variables for the 90 respective control points. We took temperature measurements for winter nests and control points by means of HOBO ® H8 Pro Series data loggers from 9 a.m. to 3 p.m. the day of the survey, placing the external sensor of the data logger 5 cm below the surface of the soil. At each of the 90 winter nests / control points we also measured the soil VWC (%) three times during the day of the survey (at 9 a.m., 12 p.m. and 3 p.m.) using a Field Scout TDR 100 / 200 sensor which measured the VWC across the surface and to a depth of up to 12 cm in the soil.

Monitoring and nest site fidelity: To determine the Argentine ant nesting preferences, we evaluated the nest site fidelity throughout 2009 and again in winter 2009 / 2010 by carrying out a bimonthly monitoring of the maintenance or abandonment of the 90 winter nests of 2008 / 2009. To check nest presence, we lifted every rock carefully to disturb the nest as little as possible.

Data analysis: We conducted descriptive analyses of the nests to gain an overall view of the physical characteristics of winter nests used by Argentine ants. We compared the following variables between abandoned and non-abandoned nests with data from winter 2008 / 2009: (1) orientation, (2) distance to the nearest nest, (3) distance to the nearest tree, (4) distance to the nearest human-made path, (5) rock surface, and (6) rock color. For comparisons we used generalized linear mixed models (GLMMs) with

Fig. 1: Photographs showing the gradual scale of rock color.

Tab. 1: Physical characteristics of the abandoned and reoccupied winter nests. GLMM (General Linear Mixed Models: P (| > χ^2|)).

Physical factor	Abandoned nests	Reoccupied nests	GLMMs
Orientation	94.45% southern	91.16% southern	*0.66*
Nearest tree (m)	0.67 ± 0.14	0.50 ± 0.08	*0.29*
Nearest path (m)	4.71 ± 0.78	5.05 ± 0.48	*0.72*
Nearest nest (m)	12.00 ± 1.94	13.19 ± 1.89	*0.73*
Rock surface (cm^2)	203.32 ± 49.83	202.99 ± 27.47	*0.99*
Rock color: light	63.63%	56.15%	*0.32*
Rock color: dark	36.37%	43.85%	

a binomial error distribution and a logit link function. We used locality as a random factor and variables (1 to 6) as fixed factors.

We also made comparisons of the canopy cover, temperature and water content of nests and control points with data from winter 2009 / 2010, using GLMMs with a Gaussian error distribution and an identity link function, and using the nest identity as a random factor and sample type (nest or control) as a fixed factor. To evaluate the environmental factors that may influence the occupation of winter nests, we performed generalized linear models (GLMs). We compared temperature and water content using a Gaussian error distribution with an identity link function in the case of temperature, or a Poisson error distribution with a log link function in the case of water content between winters 2008 / 2009 and 2009 / 2010, and a binomial error distribution with a log link function between abandoned and reoccupied nests. We also used lineal regressions to evaluate the relationship between all physical and environmental variables of nests in winter 2009 / 2010. All statistics were calculated using the R 2.12.1 for Windows package (R Development Core Team 2010) with a confidence level of 95% and a significance of $P < 0.05$. Temperature and VWC were log-transformed and canopy cover was squared-transformed to achieve normality in lineal regressions.

Results

Physical factors: Winter nests in the two invaded areas faced a southern direction in 94.45% of cases. A total of 87.78% (79 / 90) of the nests were located under rocks, of which 58.23% (46 / 79) were light colored, and the rest of nests (11 / 90) were located in the bases of trees or shrubs. The mean distance from a nest to the nearest tree was 0.51 ± 0.04 m (standard error, SE), indicating that most of the nests were located near the bases of trees or shrubs, at most 2 m away from a plant structure, regardless of the distance to the nearest human-made path (5.02 ± 0.29 m) or another nest (11.80 ± 1.04 m). There was a great variation between these distances: from 0.1 to 12 m and from 2 to 39 m, respectively. The mean rock surface was 205.45 ± 17.18 cm^2, ranging from 12 to 1176 cm^2. GLMMs performed between abandoned and reoccupied winter nests in 2008 / 2009 were not significant for all of these variables (Tab. 1). Mean canopy cover (%) was significantly higher in winter nests (60.77 ± 2.35%) than in control points (51.25 ± 2.71%) (GLMM: P (> |χ^2|) < 0.001).

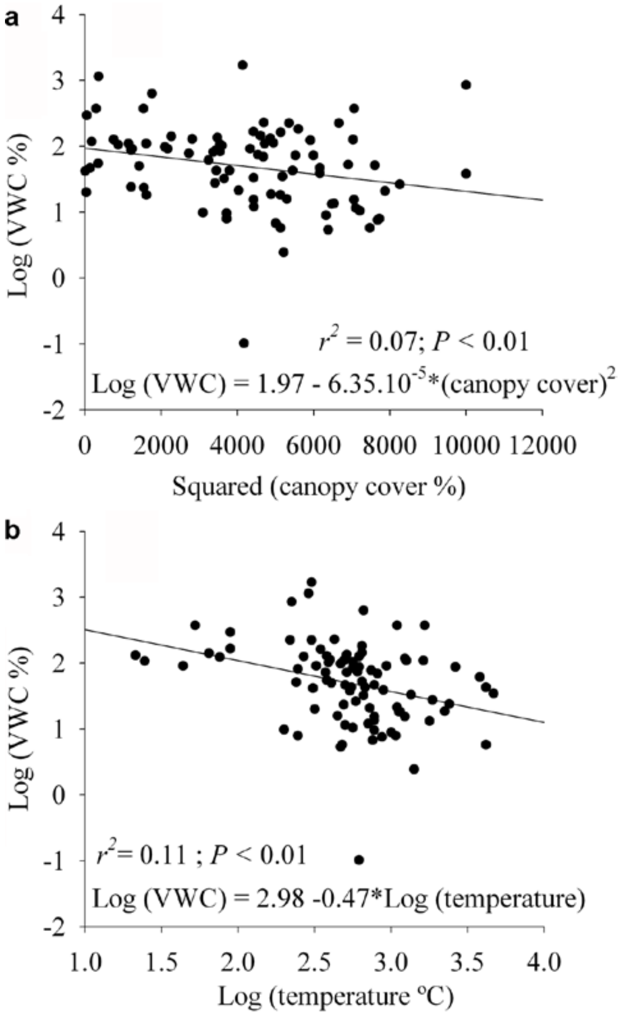

Fig. 2: Linear regressions between nest VWC (%) and (a) nest canopy cover (%) and (b) nest temperature (°C), n = 81.

Temperature and water content: The environmental characteristics of nests changed from winter 2008 / 2009 to winter 2009 / 2010. Mean nest temperature and soil water content in the first winter were significantly higher than in the second one (21.01 ± 1.01°C, 8.94 ± 0.64% for the first winter and 16.86 ± 0.75°C, 6.52 ± 0.44% for the second winter, respectively) (GLM: temperature: F = 10.89, P = 0.001; VWC: P (>|χ^2|) < 0.001). Mean soil temperatures ± SE of winter nests and control points in winter 2009 / 2010 (16.86 ± 0.75°C, 15.80 ± 0.69°C, respectively) were not significantly different from each other (GLMM: F = 3.37, P = 0.07). However, the mean soil water content ± SE was higher at control points (7.54 ± 0.52%) than at winter nests (6.52 ± 0.44%) (GLMM: P (>|χ^2|) = 0.01).

Linear regressions showed a slight relationship between winter nest soil water content and both canopy cover and nest temperature (canopy cover: r^2 = 0.07; nest temperature: r^2 = 0.11, both P < 0.01) (Fig. 2).

The range of temperatures from winter nests under light and dark colored rocks was 2.46 to 43.42°C and -1.06 to 50.11°C, respectively. A total of 26.67% (8 / 30) of dark colored rocks reached superficial nest soil temperatures up to 40 to 45°C, while light colored rocks reached only once superficial nest soil temperatures up to 40°C (Fig. 3).

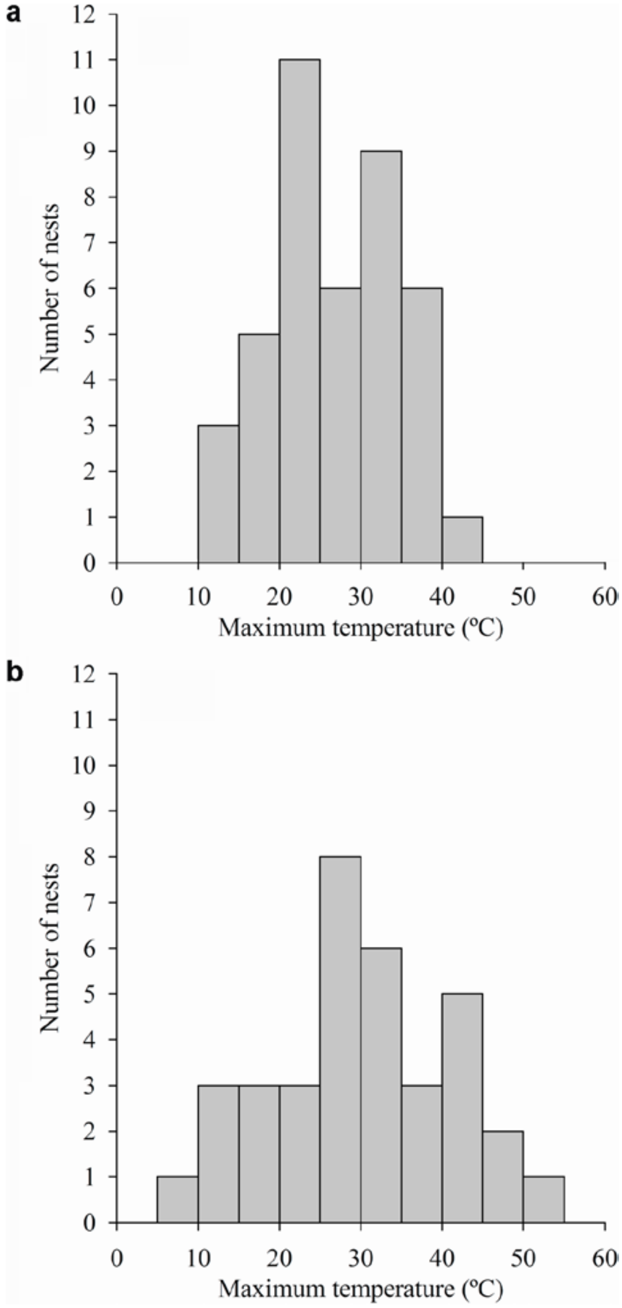

Fig. 3: Histograms of the maximum temperature reached under (a) light colored rocks (n = 46) and (b) dark colored rocks (n = 33).

Monitoring and nest site fidelity: Colonies remained the whole year in the same nest in 15.55% (14 / 90) of the cases. During 2009, the degree of nest site fidelity decreased gradually throughout the year (51.11% in spring, 38.89% in summer and 27.78% in autumn). In winter 2009 / 2010, the Argentine ant returned to most of the monitored nests, namely to 75.56% (68 / 90) of them. In spring-summer, some of the abandoned nests were colonized and used by other native species, such as *Crematogaster scutellaris* (OLIVIER, 1792) and mainly by *Plagiolepis pygmaea* (LATREILLE, 1798).

Comparison of the environmental and physical characteristics of the 22 abandoned and the 68 reoccupied nests from winter 2008 / 2009 to winter 2009 / 2010 suggests

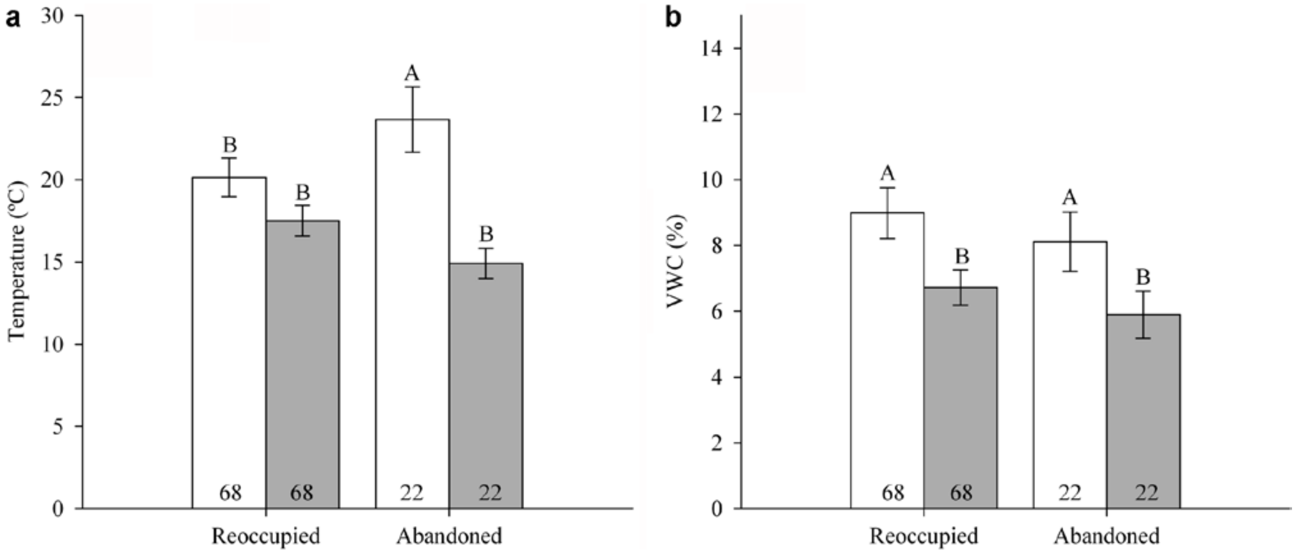

Fig. 4: a) Temperatures (°C) (mean ± SE) and b) VWC (%) (mean ± SE) of abandoned and reoccupied winter nests in 2008 / 2009 (open bars) and 2009 / 2010 (filled bars). Sample sizes are shown inside bars.

that the reoccupation of nests is related to their temperature and soil moisture. The mean temperature of nests occupied in winter 2008 / 2009 but abandoned the next winter, was higher than that of those that remained occupied in winter 2009 / 2010 (GLM: P (> $|\chi^2|$) = 0.033). Additionally, the mean temperature of nests reoccupied in winter 2009 / 2010 and those nests not abandoned in 2008 / 2009 remained the same in both years (GLM: $F = 2.39$, $P = 0.12$) (Fig. 4a). On the other hand, soil water content remained constant in all nests within the same winter (2008 / 2009 or 2009 / 2010) and decreased in occupied nests from the first (2008 / 2009) to the second (2009 / 2010) (GLM: $F = 7.38$, $P = 0.007$) (Fig. 4b).

Discussion

The Argentine ant prefers to nest in winter under rocks, mostly light colored, in places near some plant structure, thus close to a food resource with some canopy cover above the site, and facing south. Additionally, it also prefers to nest in areas where temperature and soil moisture are moderate, rather than in areas with extreme climate conditions (MENKE & al. 2007). Its nesting behavior (NEWELL & BARBER 1913, MARKIN 1970, HELLER 2004, HELLER & GORDON 2006) and its physiological characteristics (HOLWAY 1998) limit its activity and its distribution range within these areas. We found that the most important factors driving nesting location in winter were those that helped to maintain optimal conditions inside the nest. They mainly included soil moisture, temperature, factors related to the vegetation around the nest, nest orientation and rock color.

However, ROURA-PASCUAL & al. (2011) suggested that global warming and human activity is supposed to promote changes in the climatic ranges of this ant species, as they are main drivers of invasion by the Argentine ant at global scales. Moreover, ROURA-PASCUAL & al. (2004) also predicted an expansion of ant species to higher latitude areas in the future. In the context of this, we should take into account the data found on the Argentine ant abiotic preferences for nesting to predict which of the zones, where this ant species is not yet present due to low temperatures, may be susceptible to invasion in the future due to increasing temperatures with climate change. We thus expect that the nesting locations preferred by the Argentine ant will be the same in these future potential invaded zones as those found in our study area.

Winter nests were located mostly under rocks with a mean daily nest soil temperature of 16.11 to 17.61°C during mid-winter, which was slightly higher than the reported by HELLER & GORDON (2006) in California (13.1 ± 0.4°C, standard deviation). However, the general trend was similar, namely that Argentine ants were more likely to nest in warm sites, which in turn suggests that nest soil temperature influenced nest location. Additionally, these results fit within the range of temperatures found by BRIGHTWELL & al. (2010) for foraging activity during winter in northern California. This suggests that the mid-winter nest-soil temperatures found in our study area also could permit foraging activity during winter and they would thus explain the foraging activity observed during this period of the year by ABRIL & al. (2007).

Winter nest temperature is closely related to nest soil water content acting as a regulator of soil moisture, sometimes diminishing water content through evaporation and at other times maintaining it at low levels avoiding desiccation. Nest soil temperature is in turn controlled by other physical factors of the nest site, such as canopy cover, orientation and color of the rock. Canopy cover above winter nests is related to the distance at which those nests are from plant structures, which at the same time provide them a food resource near the nesting site. This would explain why the Argentine ant prefers to establish its nests closer to trees and shrubs, as HELLER & GORDON (2006) found in California. Additionally, canopy cover could have a two-side role helping to avoid extreme temperatures and also high levels of soil moisture inside nests. RETANA & CERDÀ (2000) suggested that canopy cover determines the percentage of soil exposed to the sun and the proportion of ground surface subjected to low or high temperatures. In addition, it seemed that dominant species in Mediterranean environments usually had lower thermal tolerance (CERDÀ & al.

1998, RETANA & CERDÀ 2000). In that sense, the Argentine ant, which is a well-known dominant species in natural invaded areas (HOLWAY & al. 2002), could avoid reaching extreme temperatures inside nests locating them in sites protected by the vegetation. At the same time, the canopy cover could be acting as a protection for nests against precipitation (KRUSHELNYCKY & al. 2005), optimizing moisture levels for the nest which, in fact, is what seemed to happen in the control points where the soil water content was much higher than the one from winter nests.

Furthermore, orientation and rock color may also help to protect the nest from extreme temperatures as well as to capture sunlight to provide an additional heat source regulating nest soil moisture and therefore, maintaining optimal environmental conditions inside winter nests. The main reason for the Argentine ant to nest facing south-southeast is that nests get more hours of sunlight making them warmer during winter. This could help to protect the colony from critically low temperatures that result in the failure of the colony (< 5°C longer than nine days, BRIGHTWELL & al. 2010), as well as to regulate high percentages of soil water content inside nests. In addition, this could also help to maintain some ant activity inside the nests or even to facilitate foraging when the soil temperature is below the minimum foraging threshold for *L. humile* during winter (ABRIL & al. 2007, BRIGHTWELL & al. 2010). Several authors had suggested that rocks can have thermoregulatory properties influencing the soil temperature around them (HÖLLDOBLER & WILSON 1990, NOBEL & al. 1992), protecting the colony against extreme temperatures and providing a supplementary source of heat (HÖLLDOBLER & WILSON 1990, TINAUT & al. 1999, FERNÁNDEZ-ESCUDERO & TINAUT 1999, THOMAS 2002, ROBINSON 2008, MCCAFFREY & GALEN 2011). Other ant species, such as *Proformica longiseta* COLLINGWOOD, 1978, *Rhytidoponera metallica* (SMITH, 1858) and *Formica neorufibarbis* EMERY, 1893 have preferences for a specific sun exposure, rock thickness, rock dimension and rock type (TINAUT & al. 1999, FERNÁNDEZ-ESCUDERO & TINAUT 1999, ROBINSON 2008, MCCAFFREY & GALEN 2011). Then, we could suggest that the Argentine ant has also preference for a specific nest site type, as its winter nests were built more often under light colored rocks, probably to regulate critically high temperatures (> 40 - 44°C, JUMBAM & al. 2008) and avoid excessive soil water evaporation. Light colored rocks may prevent the heating effect caused by dark colored rocks. In fact, the elevated temperatures that some of the abandoned dark colored nests reached (up to 45°C), could be a reason why those nests were left behind. This suggests that the abandonment of these nests during this period was due in part to these extreme temperatures, which forced colonies to relocate to other nests in order to find cooler and more humid locations.

In addition, we found that the Argentine ant tends to nest under a wide range of rock dimensions, from 12 to 1176 cm^2. This seems to indicate that the Argentine ant in our study area may take advantage of all rocks available for nesting regardless of their size, suggesting that they do not have preference for any particular rock size when founding their nests. In contrast, other ant species such as *Proformica longiseta* did prefer larger rocks for nesting; with regard to this result it needs to be mentioned, however, that nest rock size varied considerably due to rock size availability (50 - 250 cm^2) and that likely not a real rock selection regarding rock dimensions took place, but that *P. longiseta* rather seemed to choose suitable rocks on the basis of their thermal properties (TINAUT & al. 1999). In fact, this is what seems to happen with the Argentine ant, which was found nesting as well under a wide range of rock dimensions suggesting that nest selection would be done also *a posteriori* based on whether the properties of the rock were suitable or not for nesting in winter.

During the survey period, environmental changes occurred in the study region. The first winter (2008 / 2009) was the hottest and driest over the ten past years, and the second winter (2009 / 2010) was the coldest in the last two decades (Database of Automatic Meteorological Stations (EMA) of the Catalan Government). It is known that some ant species change their nesting sites according to the environmental conditions around the nest (TINAUT & al. 1999). The Argentine ant is one of these ants which shift its nesting behavior depending on the requirements of the moment (NEWELL & BARBER 1913, MARKIN 1970, BENOIS 1973, HELLER & GORDON 2006, HELLER & al. 2006, ABRIL & al. 2008b). The higher temperatures and dryness during the first winter could have triggered the shift of nest locations into more humid places to avoid desiccation, which could be the reason why we found higher soil moisture inside nests during 2008 / 2009 winter. Moreover, these harsh conditions could be one of the reasons why the Argentine ant abandoned some of the winter nests of 2008 / 2009 and looked for more suitable sites to nest the next winter.

Bimonthly monitoring confirmed that in a high percentage of cases (75.56%) the Argentine ant returned to the same winter nesting place year after year. A total of 15.55% of the 90 monitored nests were active the whole year. One explanation for winter nest activity throughout the year may be that nests remain active due to their size and the high density of individuals inside them, indicating the existence of mature nests that act as mother nests, as suggested by HELLER & al. (2006, 2008). These mother nests may constitute a source of queens for the colonization of new non-invaded areas in the expansion period of the species. Although we did not find any relation between rock surface and nesting site fidelity in the present study, we found a nest size - fidelity relation in other research (M. Díaz, S. Abril, M.L. Enríquez & C. Gómez, unpubl.). In that study, larger nests remained inhabited longer, some of them even for a whole year, compared to smaller nests supporting the assumption that the winter nests found active throughout the entire 2009 and in winter 2009 / 2010 in the present study were probably mother nests.

In summary, in winter the Argentine ant prefers to nest mainly under rocks. The location of these Argentine ant winter nests is mostly influenced by soil moisture and temperature, as well as factors regulating them. Thus, distance to the nearest tree, canopy cover, southern orientation and light-colored rocks help to avoid high levels of soil moisture and protect the colony from extreme temperatures maintaining optimal environmental conditions inside nests, including during winter. This information helps to improve the knowledge of the Argentine ant's nesting behavior, although further data about queen biology and physiology are needed to initiate control methods based on the reduction of queens inside winter nests in invaded natural areas.

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