ECOLOGICAL AND BIOLOGICAL STRATEGIES TAKEN BY THE ARGENTINE ANT, LINEPITHEMA HUMILE (MAYR, 1868), IN COLD SEASONS. EFFECTES OF WINTER NESTS MANAGEMENT IN NATURAL INVADED AREAS

Mireia DIAZ BUITRAGO

Dipòsit legal: Gi. 1405-2013
http://hdl.handle.net/10803/123669

Universitat de Girona

Doctoral Thesis

Ecological and biological strategies taken by the Argentine ant, *Linepithema humile* (Mayr, 1868), in cold seasons. Effects of winter nests management in natural invaded areas.

Mireia Diaz Buitrago

2012

Universitat de Girona

Doctoral Thesis

Ecological and biological strategies taken by the Argentine ant, *Linepithema humile* (Mayr, 1868), in cold seasons. Effects of winter nests management in natural invaded areas.

Mireia Diaz Buitrago

2012

Doctoral Programme in Experimental Sciences
and Sustainability

Supervisors:
Dr. Crisanto Gómez and Dra. Sílvia Abril

Memory presented for obtaining the doctoral degree at the “Universitat de Girona”

Universitat de Girona

Dr. Crisanto Gómez and Dra. Sílvia Abril, members of the Department of Environmental Sciences of the “Universitat de Girona”,

CERTIFY:

That this work, titled **"Ecological and biological strategies taken by the Argentine ant, *Linepithema humile* (Mayr, 1868), in cold seasons. Effects of winter nests management in natural invaded areas",** completed by Mireia Diaz Buitrago to obtain a doctoral degree, has been conducted under their supervision.

Dr. Crisanto Gómez	**Dra. Sílvia Abril**
Catedrático de Zoología	Investigadora Asociada
Dpto. Ciencias Ambientales	Dpto. Ciencias Ambientales
Universitat de Girona	Universitat de Girona
	Silvia A

The doctoral student

Mireia Diaz Buitrago

Girona, October 2012

A mi familia y a mi pequeño rockero.

Aquí llegaron las hormigas
vamos conquistando tierras enemigas
invisible silenciosa y simultánea
toda la invasión es subterránea […]

Hormiguero-**Calle 13**

ACKNOWLEDGEMENTS *(in Spanish)*

Las diferentes etapas de mi vida me han llevado de una forma u otra hasta donde ahora estoy, aunque sin una vocación por las hormigas en concreto, pero sí por los "bichos" (si no, ¡que les pregunten a mis padres!), finalmente empecé mi relación científica con ellas, ¡y no me arrepiento! Su estudio me ha permitido conocer, explorar, aprender y hacer míos muchos lugares y muchas gentes.

No es fácil acordarse de toda esta gente que ha pasado por mi vida y que ha hecho que esta "hormiguita" creciera y mejorara en muchos aspectos. ¡Lo intentaré!

Para empezar agradecer a mi familia el apoyo incondicional que me han brindado, no sólo durante esta etapa, sino durante toda mi vida. A mis padres, **Antonio** y **Mª Paz** por enseñarme que lo más importante en la vida es ser feliz haciendo lo que te gusta e inculcarme el amor por la naturaleza, a mi hermano **Edu** por dejarme ser una de las mujeres de su vida y ser como es, a mi **Yaya** por estar ahí siempre y ser el pilar de esta familia. A mis tías, **Berni** y **Montse**, tíos, **Juan** y **Jesús**, mi prima, **Dámaris**, mi primo **Sergi,** y a **Dani** y **Ruth**. A mi familia del otro lado del charco, **Coco**, **Norma, Paula, Ayelen** y **Ramy** por tratarme como una reina, por su inestimable ayuda física y emocional, en definitiva por acogerme y quererme como a una más. A mi pequeño "rockero" y mi gran amor, **Facu**, por todo lo que ha tenido que aguantar, levantándose a las 7 de la mañana sábados y domingos de invierno, pasando frío durante horas, aunque el mate y las galletas ¡ayudan! Por su pasión en todo lo que hace, por ser así, ¡che! ¡Te quiero!

Agradecer también a mí otra familia, la no oficial, esa a la que llamamos amigos. A los que todavía siguen a mi lado y a los que por diferentes circunstancias de la vida nuestros caminos se han separado, aunque siempre os llevo en mi corazón (**Rubén, Xiaowei, Eneritz, Marco, Luigi, Alex, Guille, Marta, Lucy, David** y **Dana**). A todos GRACIAS por interesaros siempre en lo que hacía, aunque no entendierais como podía estudiar ¡un bicho así! Y por las fiestas que nos hemos pegado en Barna y Lleida. A mis recientes pero grandes amigos en Santa Rosa, **Fran, Ezequiel, Mailén, Aldo, Nacho**, **Carlos**, y muchos más, por hacer que ésta "gallega" se sintiera como en casa a pesar de estar a ¡miles de kilómetros!

En el ámbito universitario, primeramente agradecer a **Crisanto** por confiar en mí y darme la oportunidad de volver a Girona y trabajar con este gran grupo de "pecadores". Por ayudarnos siempre y proporcionarnos siempre todo lo necesario, he aprendido mucho de ti y tu filosofía de la vida. A **Sílvia** por su gran dedicación desinteresada en esta tesis, de ella también he aprendido muchísimo en todos los aspectos, y aunque somos muy diferentes me lo he pasado en grande, sobre todo en los viajes, ¡¡¡toda una aventura cada vez que íbamos juntas!!! La verdad es que no sé si se podría haber tenido unos directores de tesis mejor. ¡¡Gracias!! Y qué decir de **Malú**! Mi compañera día tras día durante 4 años, hemos pasado mil horas juntas en el campo, pasando frío y calor, ¡¡una más que otra!! Todos los congresos que hemos compartido y lo bien que nos lo hemos pasado. Gracias también por darme siempre tu punto de vista objetivo en todo lo que hacía y ayudarme a mejorar.

También agradecer a la gente del grupo PECAT que me ha ayudado cuando lo he necesitado sin dudarlo, **Gabi, Caterina, Margarida, Núria, Pere, Pitu, David, Cape, Miguelito y J. Rost**. A la gente que no es del grupo pero que siempre estaban por ahí de una forma u otra, **Fabrice, Lluís, Meri, Sergi** y más.

Qué decir de la gente de administración las cuales me han facilitado siempre hacer todo el papeleo, **Ana Meneses, María Oliver, Montse Vélez** y las laborantes de nuestro departamento, **Gemma y Sílvia**, y las que no son de nuestro departamento, **Gemma Rustullet**, por facilitarme siempre el material que he necesitado en todo momento.

A la gente de Argentina, **Caro**, por dejar que nuestros caminos se encontraran de nuevo y facilitarme tanto las cosas allá, a la gente del EGE, **Carolina, Laura, Taty, María, Bárbara, Javier**, ¡sois una masa! y la gente de administración del departamento. A los chicos, **Eze** y **Damián**, por ayudarme en el labo con las muestras. A la gente de la Reserva Natural de Otamendi por facilitarme los permisos para el trabajo de campo.

A la gente de Sevilla, **Rapha, Xim, Fernando** y **Patro**, por permitirme entrar en sus vidas, tanto laborales como privadas, y por hacerme el calor más soportable ¡con una buena cervecita!

¡Gracias a todos y todas sin excepción!

[…en equipo se resuelve cualquier contratiempo
cuando te picamos, picamos al mismo tiempo.
Sobre nuestra unidad no debe haber preguntas
frente al peligro las hormigas mueren juntas…]

Hormiguero- **Calle 13**

Doctoral Thesis structure:

Each of the four chapters constituting this thesis has been redacted with the following scheme: introduction, material and methods, results and conclusions, with the purpose of being published in journals of the Scientific Citation Index. For this reason some contents, such as study areas or issues related to the Argentine ant, have been repeated throughout the thesis.

TABLE OF CONTENTS

SUMMARY ____ 19

RESUMEN ____ 21

GENERAL INTRODUCTION ____ 23

1. What is a biological invasion? ____ 25

2. The Argentine ant ____ 26

2.1 How did it arrive and where we can find it? ____ 27

2.2 What gives it its invasive power? ____ 29

2.3 Spatial dynamic of the Argentine ant nests ____ 31

2.4 What are the ecological effects of its introduction? ____ 32

3. How can we prevent and control the invasion? ____ 36

OBJECTIVES ____ 38

STUDY AREAS ____ 41

1. Gavarres and Cadiretes massifs, NE of the Iberian Peninsula, Spain. ____ 41

2. Reserva Natural Otamendi (RNO), Buenos Aires, Argentina. ____ 43

CHAPTER 1. Where to move when it gets cold: winter nesting sites attractive to the Argentine ant (Hymenoptera: Formicidae) in their introduced and native ranges ____ 47

1. INTRODUCTION ____ 49

2. MATERIAL AND METHODS ____ 52

2.1 Study areas ____ 52

2.1.1 Introduced range ____ 52

2.1.2 Native range ____ 53

2.2 Supercolonies in the native range: number, distribution and density of nests ____ 54

2.3 Physical factors ____ 55

2.4 Temperature and water content ____ 56

2.5 Monitoring and nest site fidelity in the introduced range ____ 57

2.6 Data analysis ____ 57

3. RESULTS ____ 58

3.1 Supercolonies in the native range: number, distribution and density of nests ____ 58

3.2 Physical factors ____ 60

3.3 Temperature and water content 63
3.4 Monitoring and nest site fidelity in the introduced range 66
4. DISCUSSION 68

CHAPTER 2. Shifts in energy investment in queens of the Argentine ant in response to different ecological contexts 77
1. INTRODUCTION 79
2. MATERIAL AND METHODS 82
2.1 Study areas 82
2.1.1 Introduced range 83
2.1.2 Native range 84
2.2 Supercolonies in the native range 85
2.3 Queen density in native and introduced ranges 85
2.4 Queen/worker thorax volume ratio in native and introduced ranges 86
2.5 Fat content of queens in native and introduced ranges 86
2.6 Daily oviposition rate of queens in the introduced range 86
2.7 Data analysis 88
3. RESULTS 89
3.1 Supercolonies in the native range 89
3.2 Queen density in native and introduced ranges 90
3.3 Queen/worker thorax volume ratio in native and introduced ranges 91
3.4 Fat content of queens in native and introduced ranges 93
3.5 Daily oviposition rate of queens in the introduced range 94
4. DISCUSSION 96

CHAPTER 3. Assessment of the Argentine ant invasion management by means of manual removal of winter nests in mixed cork oak and pine forests 103
1. INTRODUCTION 105
2. MATERIAL AND METHODS 107
2.1 Study Areas 107
2.2 Sampling methods 110
2.2.1 Nest and worker abundance 111

2.2.2 Nest size and total area of nests per plot ____ 112

2.2.3 Spatial distribution of nests ____ 112

2.2.4 Nest fidelity and nest lifetime ____ 112

2.3 Statistical Analysis ____ 113

3. RESULTS ____ 114

3.1 Nest and worker abundance ____ 115

3.2 Nest size and total area of nests per plot ____ 117

3.3 Spatial distribution of nests ____ 119

3.4 Nest fidelity and nest lifetime ____ 121

4. DISCUSSION ____ 123

CHAPTER 4. Is the submissive ant *Plagiolepis pygmaea* affected by the presence of the Argentine ant in natural invaded areas? ____ 129

1. INTRODUCTION ____ 131

2. MATERIALS AND METHODS ____ 133

2.1 Study area ____ 133

2.2 Dynamic populations: Nest density, worker abundance and nest exchange of *P. pygmaea* and *L. humile* ____ 134

2.3 Nesting site characterization: physical characteristics, temperature and soil water content ____ 136

2.4 Data analysis ____ 136

3. RESULTS ____ 137

3.1 Dynamic populations: nest density, worker abundance and nest exchange of *P. pygmaea* and *L. humile* ____ 137

3.2 Nesting site characterization: physical characteristics, temperature and soil water content ____ 141

4. DISCUSSION ____ 147

GENERAL DISCUSSION & FUTURE RESEARCH ____ 155

CONCLUSIONS ____ 167

REFERENCES ____ 173

ANNEX ____ 193

SUMMARY

RESUMEN

SUMMARY

The Argentine ant, *Linepithema humile* (Mayr, 1868), is a well-known invasive ant species that has been introduced globally into areas with Mediterranean-type climate due to human commercial activities. Argentine ant colony social behaviour is based on unicoloniality (large number of interconnected nests), polygyny (many queens per nest), and seasonal polydomy. This ant species moves its nests in response to shifts in biotic (e.g., food resources) and abiotic (e.g., temperature, soil moisture) factors, depending on the specific colony requirements in each moment. In cold seasons the spatial range of the colony contracts to create large formations and nests are combined to form the so-called “winter nests”. These winter nests are usually located in similar sites every year and it is thought they are the clue to the species’ dispersion power and the invasion of new habitats. In contrast, during hot and dry seasons, the spatial range of the colony is more disperse and distributed at random in smaller nests that are interconnected with long trails that cover a large foraging areas. Efforts to eradicate established *L. humile* populations without eliminating the queens, which constitute the reproductive power of the colony, have had little success. Methods employed have generally used wide-spectrum chemicals, which are not allowed in some protected natural areas. Thus, we propose that one possible method suitable for slowing the invasion rate on a local scale or in small recently invaded areas without chemical procedures could be the manual removal of large numbers of queens and their broods on the edge of the invasion during winter.

The aims of this thesis include: (1) determining the most preferred places for this species to build its winter nests; (2) determining the colony’s energy investment in queens; (3) knowing when and where we can better act to manage the invasion in natural invaded areas; and (4) the study of the relationships established between *P. pygmaea*, a native ant species commonly associated with *L. humile*, and the latter.

In order to know whether these ecological and biological strategies are intrinsic or were acquired when it became an invasive species, we measured the physical characteristics, temperature, and water content of the winter nests; and we analyzed queen densities per litre of nest soil, fat content in queens, and the queen/worker thorax volume ratios in nests from the native range (Natural Reserve of Otamendi (RNO), Argentina) and two invasion areas (invaded-front and invaded-centre) of the introduced range (Gavarres and Cadiretes massifs, Spain). Additionally, we characterized the Argentine ant population in the RNO by determining the number, distribution, and density of distinct supercolonies present. Furthermore, in the introduced range we monitored the Argentine ant nest site fidelity every two months, and we compare the queen oviposition rates between the invaded-front and the invaded-centre in order to determine whether there were differences in their reproductive capabilities. Once we know where to locate winter nests and which biological strategies the Argentine ant follows to assure a successful new colony founding, we can initiate methods to control the invasion based in the elimination of queens. For this purpose, first we determine the spatial dynamics of the Argentine ant nests during one year, and second, we assessed the previously proposed control method during two consecutives winters, and we analyzed the effects of this management over the long term. Finally, in order to better understand the interaction between *P. pygmaea* and the Argentine ant, we determined which factors promote the nesting site exchange, and we ascertained whether *P. pygmaea* could take advantage of the invasion in terms of resource competition. To this end, we examined the spatial dynamic of both species nests in terms of nest density, worker abundance, and nest exchange. We also characterized the abiotic components (physical characteristics, temperature, and water availability) of the nests of the two species.

Results found suggested that the Argentine ant preferred winter nesting sites that were mostly influenced by soil moisture and temperature, as well as factors regulating them. The closeness to trees or shrubs that provide some cover, southern orientations (in both native and introduced areas), and the shelter of rocks (in the introduced range) help to avoid high levels of soil moisture and protect the colony from extreme temperatures while maintaining optimal environmental conditions both inside the nests and for colony activity during the winter. We also confirmed that in the introduced range the Argentine ant has a certain fidelity to its winter nesting sites, returning to the same places year after year. Moreover, native supercolonies also seem to follow a yearly pattern of fusion-fission of nests and *L. humile* nesting behaviour in winter is comparable to it operates in invaded areas, locating their winter nests in largely similar (though no identical) sites. On the other hand, we found biological differences between native and introduced ranges in terms of the Argentine ant energy investment in queens. Colonies in the native range have a greater number of queens, and they are smaller in size than in both invaded zones. They also have the highest fat content, and the largest workers. Colonies from the invaded-front, however, also have a greater number of queens, and they are larger than in the invaded-centre, but with the same fat reserves and worker size. Thus, the Argentine ant queens in the native range could be physiologically and

morphologically adapted to an independent mode of colony founding, while those in the introduced areas seem to occupy a halfway point between independent and dependent, as morphologically queens are qualified to carry out independent colony founding, but physiologically they are not. In regard to the spatial dynamic of nests, during the first year all *L. humile* colonies tended to follow an annual cycle of contraction and dispersion, with a decrease in the number of nests as we approach to the invaded-front. The extirpations conducted only had an effect at plots located in the invaded-front, and promoted smaller, less lasting and aggregated nests, as well as a decrease in the abundance of workers. However, nests and workers experienced a decrease during the two first winters but a recovery in the third.

Against this background, we suggest that the winter nesting behaviour of the Argentine ant seems to be intrinsic, and that the success of the Argentine ant as an invasive species does not rely on a shift in social organization, nor on a shift in its mode of nesting associated with the introduction to new habitats. Furthermore, differences in energy investment found in queens seem to respond to the different ecological contexts between the three zones studied (native, invaded-front and invaded-centre). Thus, it could represent a shift in the colony's biological strategies to become an invasive species in the introduced range. In such scenario, we suggest to better focus the management of the invasion by means of manual removal of nests in winter, at the edge of the front, and do it yearly. This practice could help us to achieve significant results and to keep the number of nests, and thus the expansion of the Argentine ant, at a low rate.

Finally, as to the relationship established between *P. pygmaea* and *L. humile*, the results suggest that *P. pygmaea* seems to be affected by the presence of *L. humile* in the invaded-front, reducing its presence in these areas. However, the plasticity in nesting behaviour and colony activity permits this tiny ant to coexist in these invaded-front areas where the Argentine ant is present. On the contrary, in the invaded-centre *P. pygmaea* seems to take advantage of the presence of the Argentine ant. The former increases its presence in these areas thanks to the fact that *L. humile* reduces the competitive pressure on it by retracting other native ants, and also because of the existence of a certain degree of habituation between these two species.

RESUMEN

La hormiga argentina, *Linepithema humile* (Mayr, 1868), es una de las especies invasoras de hormigas introducidas a nivel mundial en zonas de clima mediterráneo debido a las actividades comerciales humanas. El comportamiento social de sus colonias se basa en la unicolonialidad (muchos nidos interconectados), la poliginia (muchas reinas por nido) y la polidomia estacional. Esta especie mueve sus nidos en respuesta a cambios en diferentes factores bióticos (ej., recursos alimenticios) y abióticos (ej., temperatura, humedad del suelo) en función de las necesidades específicas de las colonias en cada momento. Durante las estaciones frías, el rango espacial de la colonia se contrae en grandes formaciones y los nidos se combinan para formar los llamados "nidos de invierno". Estos nidos de invierno están generalmente ubicados bajo piedras, en sitios similares cada año y se cree que son la clave para la dispersión de la especie y la invasión de nuevos hábitats. Por el contrario, durante las estaciones cálidas y secas, el rango espacial de la colonia es más disperso y está distribuido al azar en pequeños nidos interconectados mediante largas pistas que cubren grandes áreas de forrajeo. Los esfuerzos por erradicar las poblaciones establecidas de *L. humile* sin eliminar las reinas, quienes tienen el poder reproductivo de la colonia, han tenido poco éxito. Los métodos que se emplean generalmente son productos químicos de amplio espectro que no están permitidos en áreas naturales protegidas. Por eso, sugerimos que un posible método de control, sin uso de químicos y adecuado para ralentizar el ritmo de invasión a escala local o en pequeñas áreas recientemente invadidas, podría ser la extirpación manual de reinas y su progenie en el frente de la invasión durante el invierno.

Los objetivos de esta tesis son: (1) la determinación de los lugares preferidos por esta especie para construir sus nidos de invierno; (2) la determinación de la inversión energética en reinas de las colonias; (3) saber cuándo y dónde actuar para poder mejorar la gestión de la invasión en áreas naturales; (4) y el estudio de las relaciones establecidas entre *P. pygmaea,* una de las especies de hormiga nativa más frecuentemente relacionada con *L. humile*, y ésta última.

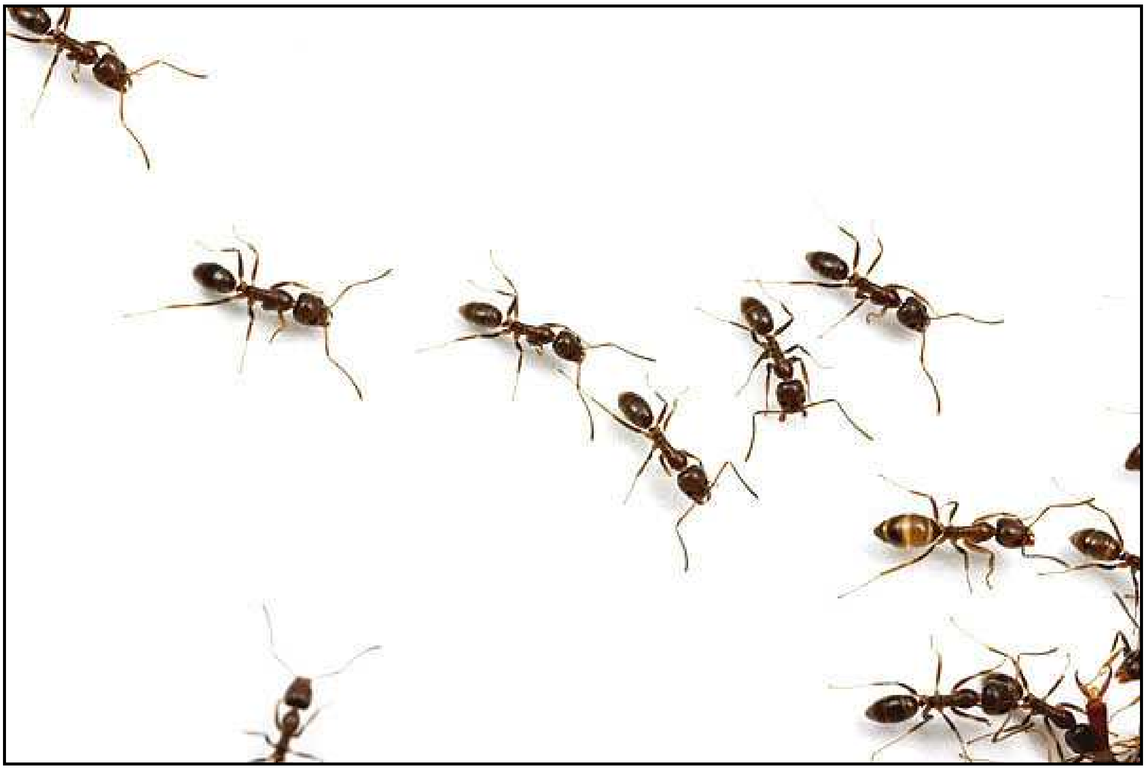
Con el fin de saber si las estrategias ecológicas y biológicas tomadas por *L. humile* son intrínsecas, o se han adquirido con el paso a especie invasora, se analizaron las características físicas, la temperatura y la humedad de los nidos de invierno, así como las densidades de reinas por litro de nido, el contenido en grasas de las reinas y la relación del volumen del tórax reina/obrera en los nidos de la zona nativa en la Reserva Natural de Otamendi (RNO), Argentina y en dos zonas invadidas (frente y centro de la invasión) de los macizos de las Gavarres y Cadiretes, España. Además, se caracterizó la población de hormiga argentina en la RNO determinando el número, la distribución y la densidad de las distintas supercolonias presentes. En la zona invadida también se realizó un seguimiento de la fidelidad de los nidos cada dos meses y se compararon las tasas de ovoposición de las reinas entre el frente y el centro de la invasión, para determinar si existen diferencias en sus capacidades reproductivas. Una vez que sabemos dónde ubicar los nidos de invierno y qué estrategias biológicas siguen las colonias para garantizar el éxito de nuevas fundaciones, podemos iniciar métodos para el control de la invasión basados en la eliminación de las reinas. Para ello, en primer lugar, se determinó la dinámica espacial de los nidos de *L. humile* durante un año, y en segundo lugar, se evaluó el método de control propuesto anteriormente durante dos inviernos consecutivos y se analizaron los efectos de esta gestión a largo plazo. Por último, para entender mejor la interacción entre *P. pygmaea* y la hormiga argentina, se determinaron los factores que promueven el intercambio de nidos entre estas dos especies, y si *P. pygmaea* podría aprovechar la invasión de la hormiga argentina en lo referente a la competencia por los recursos. Para ello, se analizó la dinámica espacial de los nidos de ambas especies en términos de densidad de nidos, abundancia de obreras e intercambio de nidos. También se caracterizaron los factores abióticos (características físicas, temperatura y humedad) de los nidos de las dos especies.

Los resultados encontrados sugirieron que los sitios preferidos por la hormiga argentina para anidar en invierno están principalmente influenciados por la humedad y la temperatura del suelo, así como por factores que pueden regularlas. La cercanía a árboles o arbustos que les proporcionan cierta cobertura vegetal, orientaciones hacia el sur (en ambas zonas nativa e invadida) y el refugio que proporcionan las piedras (en la zona invadida), ayudan a evitar altos niveles de humedad en el suelo y a proteger a la colonia de temperaturas extremas, manteniendo unas condiciones ambientales óptimas en el interior de los nidos para la actividad de la colonia durante el invierno. También, se confirmó que la hormiga argentina en la zona invadida tiene una cierta fidelidad por sus nidos de invierno volviendo al mismo lugar año tras año. Por otra parte, las supercolonias nativas también parecen seguir un patrón anual de la fusión-fisión de los nidos teniendo un comportamiento de anidación en invierno similar a las áreas invadidas y localizando los nidos de invierno en sitios del mismo tipo. Por otro lado, se encontraron diferencias biológicas entre la zona nativa e invadida con respecto a la inversión energética en reinas. Las colonias en la zona nativa contenían un mayor número de reinas y de menor tamaño que en ambas zonas invadidas (frente y centro de la invasión). El contenido en grasas de las reinas en la zona nativa también era mayor, y además tenían unas obreras más grandes que en las dos zonas invadidas. Mientras que las colonias del frente de la invasión contenían más reinas y eran más grandes que las del centro de la invasión, pero con las mismas reservas de grasas y el mismo tamaño de obreras. Por lo tanto, las reinas en la zona nativa podrían estar fisiológica y morfológicamente adaptadas a un modo de fundación independiente de la colonia, mientras que en las zonas invadidas podrían encontrarse en un punto medio entre fundación independiente y dependiente, ya que las reinas están morfológicamente cualificadas para llevar a cabo una fundación de la colonia independiente, pero fisiológicamente no. En lo que respecta a la dinámica espacial de nidos, todas las colonias de *L. humile* durante el primer año tendían a seguir un ciclo anual de contracción-dispersión con una disminución en el número de nidos a medida que nos acercábamos al frente de la invasión. Las extirpaciones realizadas sólo tuvieron efecto en las parcelas ubicadas en el frente de la invasión, y promovieron nidos más pequeños, menos duraderos y agregados, así como una disminución en la abundancia de las obreras. Aunque los nidos y las obreras experimentaron un descenso durante los dos primeros inviernos, se observó una pronta recuperación en el tercer invierno.

Bajo este contexto, se podría decir que el comportamiento de anidamiento en invierno de la hormiga argentina parece ser intrínseco y el éxito como especie invasora no se basa en un cambio en la organización social ni en un cambio en su modo de anidación asociado a la introducción en nuevos hábitats. Por otra parte, las diferencias en inversión energética en reinas parecen responder a los diferentes contextos ecológicos de las tres zonas estudiadas (nativa, frente y centro de la invasión). Por lo tanto, podría haber un cambio en las estrategias biológicas de la colonia para convertirse en una especie invasora. Ante tal situación, se sugiere centrar la gestión de la invasión por medio de la extirpación manual de los nidos, en invierno, en el borde del frente de la invasión y además hacerla cada año. Esta práctica nos puede ayudar a tener resultados muy significativos y a mantener el número de nidos, y en consecuencia la tasa de expansión de esta especie a bajos niveles.

Por último, en cuanto a la relación establecida entre *P. pygmaea* y *L. humile*, los resultados sugieren que la presencia de la hormiga argentina en el frente de la invasión parece perjudicar a *P. pygmaea* disminuyendo su presencia en estas áreas. Sin embargo, la plasticidad de esta pequeña hormiga en el comportamiento de anidación y en la actividad de la colonia, le permiten convivir con *L. humile* en el frente de la invasión. Contrariamente, en el centro de la invasión la presencia de la hormiga argentina parece beneficiar a *P. pygmaea.* Esta última aumenta su presencia en estas zonas gracias a que *L. humile* disminuye la presión de la competencia sobre ella retrayendo a las otras hormigas nativas, y a la existencia de un cierto grado de habituación entre ambas especies.

GENERAL INTRODUCTION



(picture: Alex Wild)

GENERAL INTRODUCTION

1. What is a biological invasion?

The result of the “globalization” process that we have been experiencing since the mid-twentieth century as a consequence of the fusion of markets, societies and cultures, is that we live in a world almost without boundaries. The human capacity to eliminate geographical barriers has accelerated exponentially the introduction of exotic species, whether intentionally or accidentally, during recent decades (Mack et al. 2000).

In such a scenario, few if any areas remain sheltered from the immigration of different exotic species, which can become invasive when they finish their naturalization and expansion process far from their introduction focus (Vilà et al. 2008).

A biological invasion is the result of different steps through which these types of species have to pass, from the moment they are introduced out of their natural environment until their complete establishment and expansion in new geographical areas (Williamson & Fitter 1996, Mack et al. 2000, Kolar & Lodge 2001). The percentage of non-native species in a particular country as a proportion of the total fauna and flora may comprise from a few percent to more than 20% of the total (Vitousek et al. 1996). Fortunately, however, it has been estimated that only one in a thousand introduced species becomes invasive (Williamson & Fitter 1996).

This free movement of potentially invasive species has several ecological and economic consequences within ecosystems in which they establish themselves (Mack et al. 2000, Pimentel et al. 2000). They cause biodiversity loss, which changes ecosystem functionality and alters the composition and structure of the invaded community (Clavero & Brotons 2010). These species homogenize the ecosystem they invade as a result of the replacement of local biota. They also promote the loss of endemic local species, loss of harvest and domestic animal production, as well as produce several types of infrastructural damage (McKinney & Lockwood 1999). These species can have either a direct or indirect negative impact on public health, and consequently, on society in general. Moreover, they produce innumerable economic losses due to the cost of the effective target management of the eradication of the invasive species.

Invasive species are represented by numerous taxonomic groups (www.issg.org/database/welcome). One of the groups more easily introduced accidentally via human activity is the phylum Arthropoda (Vitousek et al. 1996). Among them, there is a great diversity of invasive ant species considered to be plagues.

In the Iberian Peninsula twelve non-native ant species have been detected. Two of these, *Lasius neglectus* (Van Loon, Boomsma & Andrasfalvy 1990) and *Linepithema humile* (Mayr 1868), are invasive and pose a potential threat for native animal and plant communities in affected areas (Espadaler & Collingwood 2000). The latter, known as the Argentine ant, is the main object of study of this thesis.

2. The Argentine ant

The Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae: Dolichoderinae), is an invasive ant species. In Mediterranean and/or subtropical-type climates it is considered to be one of the urban, agricultural and natural environmental plagues with the greatest worldwide expansion (McGlynn 1999). This "modest" ant has been catalogued as one of the 100 worst invasive species in the world by the ISSG (Invasive Species Specialist Group, www.issg.org/database/welcome). This species is a small brown monomorphic (without caste division within workers) ant. Workers measure about 2-3 mm and queens about 4-6 mm long (Wild 2004) **(Figure 1).**

Figure 1. Argentine ant worker in frontal, lateral and dorsal view (source: www.antweb.org, pictures: April Nobile and Eli M. Sarnat).

2.1 How did it arrive and where we can find it?

Native to South America, in its natural habitat the Argentine ant is distributed along the basin of the Paraná River (Tsutsui et al. 2001, Wild 2004), from the south of Brazil, through Paraguay and Uruguay to the northeast of Argentina. This ant species has been introduced globally due to human commercial activities, and with the exception of Antarctica only, is now present on six continents and on several oceanic islands such as Japan, New Zealand, and Hawaii (Hölldobler & Wilson 1990, Passera 1994, Suarez et al. 1998, Suarez et al. 2001, Roura-Pascual et al. 2010) (**Figure 2**). This ant is often associated with human disturbance (Suarez et al. 1998); however, it has the ability to invade undisturbed natural areas with a low level of anthropization (Holway 1998a, Gómez et al. 2003).

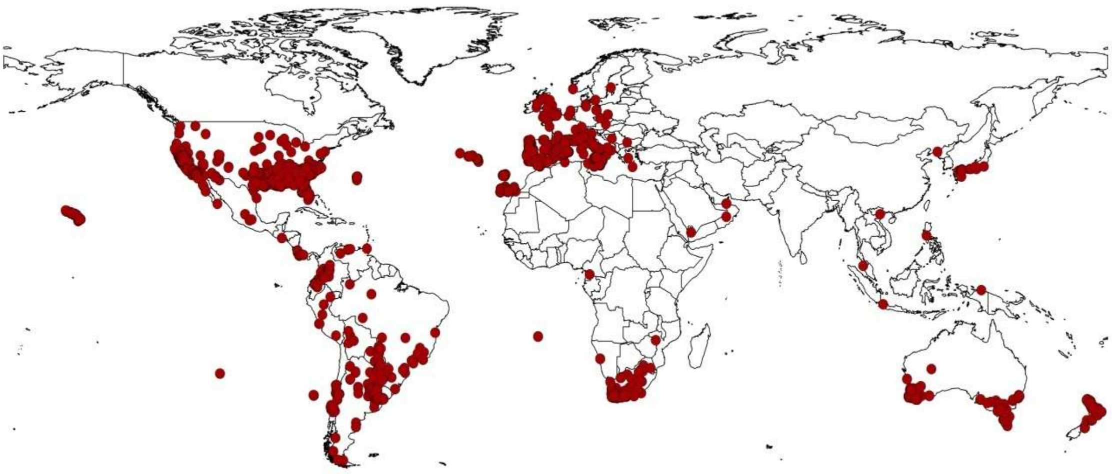


Figure 2. Argentine ant global-scale distribution (source: Roura-Pascual et al. 2010).

The presence of the Argentine ant in the Iberian Peninsula was first detected in 1907 in Portugal (Martins 1907) and in Spain, on the Valencian coast, probably in 1919 (García-Mercet 1921, Font de Mora 1923). Nowadays the Argentine ant is present along the entire coastal band of the Iberian Peninsula, on the Balearic Islands and the Canaries Archipelago **(Figure 3)**. Its distribution seems to be limited to temperate and humid climates (Holway et al. 2002a) as it is heavily influenced by temperature and water availability, factors which have a strong influence on some of the species' biological traits, e.g., queen oviposition rate (Benois 1973, Abril et al. 2008a), brood development rate (Newell & Barber 1913, Benois 1973, Abril et al. 2008a), and foraging effectiveness (Markin 1970, Human & Gordon 1999, Witt & Giliomee 1999, Holway et al. 2002a, Abril et al. 2007, Jumbam et al. 2008). Therefore, its sporadic presence on the Cantabrian coast, where the climate is colder and more humid, and in the centre of the Iberian Peninsula, where the climate is very dry, seems to be because in these locations the Argentine ant is associated with urban areas whose modified local climatic conditions may be suitable for the species' survival (Espadaler & Gómez 2003).

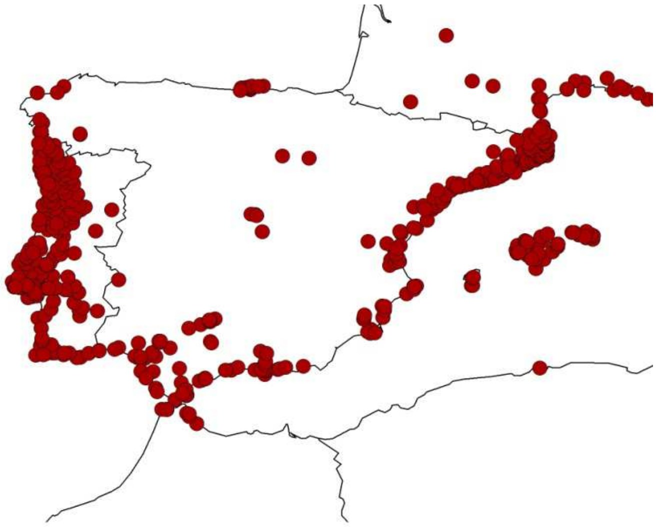


Figure 3. Argentine ant distribution in the Iberian Peninsula and Balearic Islands (source: Roura-Pascual et al. 2010).

2.2 What gives it its invasive power?

There are several biological and behavioural characteristics which have been key to the success of the Argentine ant as an invasive organism. One of these characteristics is the social behaviour of colonies, which is based on unicoloniality (Passera 1994). This type of social organization is present in both native and introduced ranges (Vogel et al. 2009), and is characterized by the formation of large colonies with a large number of interconnected nests, with workers and queens moving freely among them (Heller 2004, Heller & Gordon 2006). These large formations, known as "supercolonies", display no intercolonial aggression as a result of a low genetic differentiation within colonies (Heller et al. 2008a). In the introduced range this lack of intercolonial aggression provides the Argentine ant with many advantages. It contributes greatly to the increase in its population density, and is responsible for its dispersal success as an invader (Heller 2004). Its numerical dominance improves its competitiveness both in terms of resource exploitation (exploitation competition), and the level of competition with native ant species (interference competition) (Heller 2004).

On the other hand, Argentine ant colonies in the introduced range are highly polygynous (they contain a large number of queens) (Keller 1995) and reproduce by budding. This kind of reproduction consists of the abandonment of the original colony by a fraction of its population–containing some workers and one or more queens–which moves through the soil surface over short distances to find new sites, and results in the gradual dispersion of the colony. Queens are inseminated prior to dispersal and they are not involved in a mating flight out of the nest (Suarez et al. 1998). In the case of both global and regional introduced distributions, the establishment of new Argentine ant foci depends on long-distance jump-dispersal events through human-mediated transport (Suarez et al 2001), but once settled into a new area, expansion occurs mainly by budding.

Other features that enhance its invasive success in introduced areas are its omnivorous diet, which includes nectar, insects, seeds, carrion, honeydew secreted by hemipterans (Markin 1970, Suarez et al. 1998), and the lack of natural predators, parasites and other native ant competitors (Orr et al. 2001, Tsutsui et al. 2001, Holway et al. 2002b, Reuter et al. 2005). In addition, *L. humile* is known to have a lack of hibernation period in areas under invasion (Benois 1973, Holway 1998a, Abril et al. 2007), allowing it to profit from the absence of activity in other native ant species that could be competing with it for nesting sites or food resources. In winter, due to the warm local microclimate of invaded Mediterranean-type climate areas, and with an optimal range of temperature for foraging activity during daytime hours (Abril et al. 2007, Brightwell et al. 2010), this ant species is capable of colonizing new areas and forcing the native species in physiological stop to retreat.

Thus, the only difference between colonies in both ranges is their size, which is much smaller in the native range (Vogel et al. 2009). Vogel et al. (2009) also suggested that the success of the Argentine ant as an invasive species is therefore not the result of a shift in social organization associated with its introduction into new habitats, rather it is more likely explained by the characteristics it develops in its native range, combined with the ecological release from predators, parasites, and competitors that follows introduction into a new habitat (Orr et al. 2001, Tsutsui et al. 2001, Holway et al. 2002b, Reuter et al. 2005, Vogel et al. 2009).

2.3 Spatial dynamic of the Argentine ant nests

Due to its unicolonial structure, Argentine ant colonies present a seasonal polydomy (Heller et al. 2008a). This means that the nesting behaviour of Argentine ants changes according to biotic factors (e.g., surrounding plant structures, canopy cover, etc.) and abiotic factors (e.g., temperature, soil water content etc.) and differs from summer to winter (Heller et al. 2006). These factors are the key to its establishment in new areas (Roura-Pascual et al. 2004, 2006, Jumbam et al. 2008). The Argentine ant is heavily influenced by temperature and water availability and it has significantly higher rates of water loss and cuticular water permeability than native ant species adapted to hot and dry Mediterranean environments (Schilman et al. 2007). In fact, Argentine ant 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. Therefore, the Argentine ant moves its nest in response to seasonal shifts in environmental conditions or food availability, depending on the specific colony requirements at that moment (Heller et al. 2006). During the cold season in Mediterranean-type climate areas the spatial range of the colony contracts and nests are combined to form the so-called “winter nests” (Newell & Barber 1913). In contrast, during the hot and dry season the spatial range of the colony is more dispersed and distributed at random in smaller nests which are interconnected with long trails that cover large foraging areas **(Figure 4).** In addition, these seasonal shifts produce a fluctuating invasion front, and consequently a fluctuating impact on native species from these zones (Heller et al. 2006, M.L. Enríquez, S. Abril, M. Diaz & C. Gómez, unpubl.).

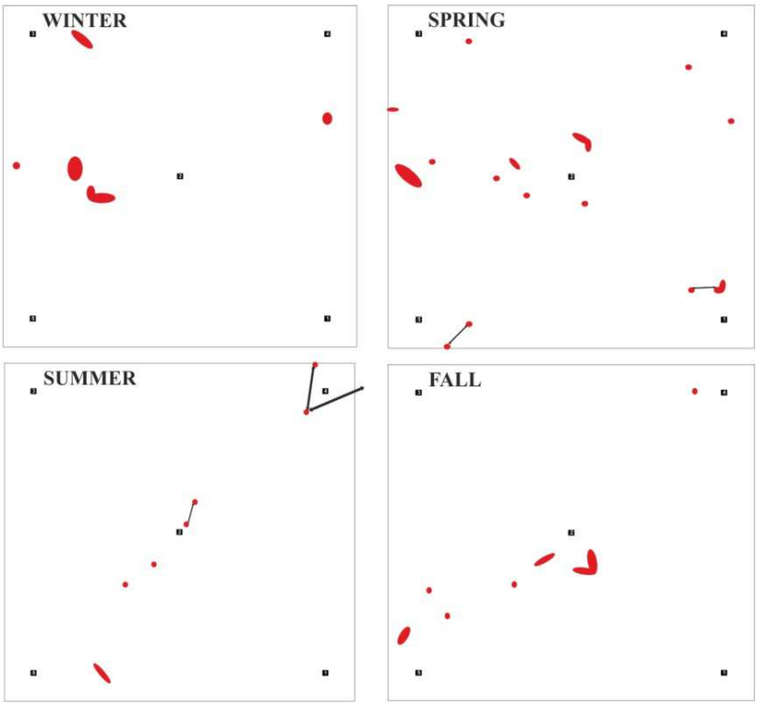


Figure 4. Schematic illustration of the seasonal distribution of nests at one sampled plot. Red spots represent nests; the size of the spot represents the nest size. Black lines indicate trails. Black squares represent pitfall traps within each plot.

2.4 What are the ecological effects of its introduction?

The aggressive behavior (Carpintero & Reyes 2008) and the numerical dominance of the Argentine ant in introduced areas has a negative impact on native ant species, arthropod communities (Human & Gordon 1996, Holway 1998b, Suarez et al. 1998, Oliveras et al. 2005), ant-vertebrate interactions (Suarez et al. 2000, Estany-Tigerström et al. 2010), and ant-plant relationships (Bond & Slingsby 1984, Visser et al. 1996, Gómez & Oliveras 2003, Gómez et al. 2003, Blancafort & Gómez 2005, Rodríguez-Cabal et al. 2009), thereby causing a drastic decrease in the biodiversity of the invaded areas. In fact, in Mediterranean ecosystems there are many plants whose seeds are dispersed by native ants, and the presence of the Argentine ant contributes to the disappearance of both native ants and plant species, and consequently to the disruption of this mutual dispersion **(Figure 5a)** (Gómez & Oliveras 2003, Gómez et al. 2003, Blancafort & Gómez 2005, Rodríguez-Cabal et al. 2009). Moreover, the presence of the Argentine ant may also influence the reduction of certain vertebrate populations, as has occurred with the California horned lizard, which feeds mainly on granivorous ants (Suarez et al. 2000), or with the canopy-foraging foliage-gleaning birds in the northeast Iberian Peninsula, whose young have diminished in invaded areas due to the decrease of available caterpillars extracted by this ant species (Estany-Tigerström et al. 2010). Thus, the Argentine ant may have a negative impact on the trophic web through its effects on the availability of arthropod prey for insectivorous vertebrates **(Figure 5b and 5c)**.

This species can also cause important indirect damage to crops, as it tends honeydew-excreting hemipterans and can disrupt the activity of their natural enemies **(Figure 5d)**. It has been reported that in vineyards the Argentine ant alters the biological control of hemipteran plagues, interfering aggressively against parasites and predators that feed on them (Daane et al. 2007).

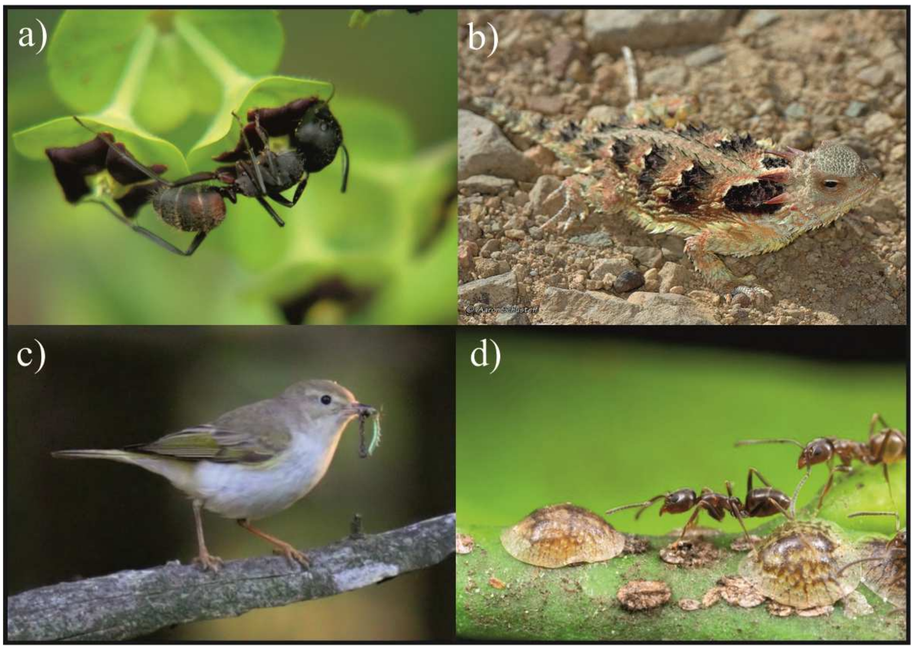


Figure 5. (a) Mutualism between *Euphorbia characias* L. and *Camponotus cruentatus* (Forel 1890) (source: Acideformik.com, picture: Cekiki), (b) Coast horned lizard (*Phrynosoma coronatum* (Blainville 1835), source: CalPhotos project, BSCIT, picture: Aaron Schusteff), (c) *Phylloscopus bonelli* (Vieillot 1819) (source: IBC, picture: Juan Lacruz Martín) and (d) workers of the Argentine ant tending scale insects on an orange tree (source: myrmecos.net, picture: Alex Wild).

In its native range the Argentine ant is actually not able to dominate the natural communities found there due to the additional pressure of intraspecific competition, natural predators, and parasites (Tsutsui et al. 2001, Holway et al. 2002b). Hence, *L. humile* coexists with a diverse community of ants (Suarez et al. 1999); approximately 51 ant species (Cabrera 2009) compete against it, some of which are highly dominant.

However, in the invaded natural areas of the northeast Iberian Peninsula we were able to find some species coexisting with *L. humile* (M.L. Enríquez, S. Abril, M. Diaz & C. Gómez, unpubl.). One of the species present which has not been displaced by the Argentine ant is *Plagiolepis pygmaea* (Latreille 1798) (Oliveras et al. 2005, Abril & Gómez 2009). It seems that the ability of *P. Pygmaea* to avoid confrontation and the use of extremely submissive behaviour to appease opponents promotes the lack of aggression from the Argentine ant towards it, which contributes to the coexistence of the two ant species (Abril & Gómez 2009).

Plagiolepis pygmaea (Hymenoptera: Formicidae: Formicinae), is a tiny dark-coloured monomorphic (without caste division within workers) ant; workers measure <3 mm (**Figure 6**).

Figure 6. *Plagiolepis pygmaea* worker in frontal, lateral and dorsal view (source: www.antweb.org, pictures: April Nobile).

We were able to find this ant species distributed along the south of Europe, from Spain to Austria, and usually in arid areas with little vegetation.

P. pygmaea has an omnivorous dietary regime, but it has a preference for sugary liquids, which means that workers feed on nectar and honeydew secreted by hemipterans (Bernard 1968, Suay-Cano et al. 2002). It can also be found to prey on other insects (Serrano et al. 1987), and has been cited as a constituent of the sarcosaprophagous community of Mediterranean ecosystems in Murcia (Martínez et al. 2002).

This ant species is polygynous (several queens per nest). Mercier et al. (1985) found an average of 17 queens per nest and suggested that the individual productivity of each queen weight varies according to its appeal to workers and the number of individuals in the colony. Additionally, *P. pygmaea* practices convenience polyandry (multiple mating) in order to increase the probability of the insemination of queens and assure colony survival (Trontti et al. 2006). Reproduction of this species occurs by budding (intranidal mating), though the queens of the colony are also able to found an independent colony by taking reproductive individuals outside the range of related colonies (Passera 1969, Trontti et al. 2006).

On the other hand, *P. pygmaea* constitutes polydomous colonies without aggression. These are comprised of many interconnected nests, between which queens, workers and brood are exchanged (Passera 1963). Additionally, the number of nests per colony varies seasonally, and they change nest location with shifts in environmental conditions. In spring colonies split into numerous nests, remaining in close proximity to one another, and in late summer nests are fused with other colony subunits in large formations known as “winter nests”, thus reducing the level of polydomy for overwintering (Passera et al. 2001).

3. How can we prevent and control the invasion?

The best way to reduce the impact of *L. humile* in natural ecosystems is to avoid its introduction in new non-invaded areas. For this purpose, protocols need to be in place to minimise the chances of transporting them to key conservation areas, and an early warning monitoring scheme established in areas identified as high risk (Harris 2002).

When prevention protocols do not work or are overcome, as has occurred in many places around the whole world, there is a range of options available or under development to control *L. humile*, although most of them do not have the ability to eradicate entire established populations in invaded natural areas. Moreover, the probability of success in eradicating an invasive species decreases with an increase in its distribution range (Myers et al. 2000). For this reason the eradication of the Argentine ant in natural habitats over hundreds of hectares is practically impossible. In such places, the most sensible way to control the invasion is to slow the rate of spread in order to limit its establishment in non-invaded areas, and thus its negative impact on the ecosystem.

The most common control method used is toxic baits (Krushelnycky & Reimer 1998a, 1998b). This method reduces worker populations but seems to fail at killing the queens, which hold the reproductive power of the colony. In consequence, colony foraging activity rates recover rapidly after the application of the treatment (Krushelnycky & Reimer 1998a, 1998b). Bait with sucrose and boric acid has also been assessed and has shown to be effective in the control of queens in the laboratory when the bait was continuously available (Hooper-Bui & Rust 2000, Klotz et al. 2000), though there is no information on its effect in natural areas. However, the use of chemicals is not allowed in some natural areas of special interest as they are wide-spectrum products and can have a negative impact on other arthropods.

Against this background, several authors tried different ways to disrupt the dynamics of the Argentine ant colonies using biological procedures. These methods may be able to be used as a way to control *L. humile* invasions in the future. Some have attempted to disrupt the foraging activity of workers with a synthetic pheromone (Suckling et al. 2008, Nishisue et al. 2010, Suckling et al. 2010), which seems to suppress worker recruitment in the short term (Tanaka et al. 2009), but the long-term effect is very weak when the density of the target pest is high (Cardé 1990, Nishisue et al. 2010). Nishisue et al. (2010) concluded that the combination of toxic baits and pheromones, along with native ant competitors, could be an effective way to suppress resource acquisition by Argentine ants. Additionally, Liang and Silverman demonstrated that the Argentine ant displays an intracolony aggression when different colony fragments are reared on different diets (Liang & Silverman 2000). Diet dissociation seems to change cuticular hydrocarbon profiles, altering prevailing nest-mate recognition cues in the Argentine ant and effectively creating separate colonies (Silverman & Liang 2001).

We suggest another possible method of control based on the extirpation of the Argentine ant winter nests. The manual removal of large numbers of queens and their broods in the advancing invasion front in winter (the period of maximum queen densities, Abril et al. 2008b) could be a way to weaken its expansion without the use of chemical procedures. The systemic elimination of queens during this period might offer a degree of resistance to the invasion, and hence, to its expansion into non-invaded areas on a local scale or in small and recently invaded areas of natural interest.

OBJECTIVES

As efforts to eradicate *L. humile* have had little success and the use of chemicals is not allowed in some natural areas of special interest (particularly wide-spectrum products that could have a negative impact over other arthropods), the most reasonable way to control the invasion is to slow the spread rate and to limit its establishment in other non-invaded areas and the consequent negative impact on the ecosystem. One possible method of control could be the manual removal of large numbers of queens and their broods on the edge of the invasion during winter, which is the period of maximum queen density inside nests (Abril et al. 2008b). This method could be suitable for slowing the invasion rate on a local scale or in small and recently invaded areas of natural interest.

With this in mind, the correct location of Argentine ant winter nests is an important key in effective invasion management based on the extraction and elimination of queens and their brood in the advancing front. In **CHAPTER 1** we determine the nesting preferences of *L. humile* in winter in both introduced and native ranges to discover whether this winter nesting behaviour is intrinsic or acquired through becoming an invasive species. Thus, we hope to make the location of these potential nesting places more accessible, and to permit a more efficient site management to be carried out based on the elimination of queens and their brood in invaded areas of natural interest.

On the other hand, in order to better understand the invasion process in natural environments and to know whether the energy investment of the colony has changed in the process of becoming an invasive species, we attempt to compare different traits of the biology and physiology of the queens in nests situated in the native range and in the introduced range, at the forefront of expansion (in contact with the native ant population), and in the invaded zone (where the Argentine ant is almost the only ant present). For this purpose, in **CHAPTER 2** we examine differences in Argentine ant queen densities, in the fat content of queens, and in the queen/worker thorax volume ratio in nests from the native range and the two areas of the introduced range. Additionally, we compare queen oviposition rates between invasion sites (contact and invaded zones) in order to determine whether there are differences in their reproductive capabilities.

Once we had characterized the winter nest and localized the possible areas in which the Argentine ant tends to nest during that period, we conducted a systemic elimination of nests in the hope that it might offer a degree of resistance to the invasion, and hence, to its expansion into non-invaded areas. This is what we assess in **CHAPTER 3.** From here, we attempt to discover how the spatial dynamics of the Argentine ant nests change seasonally in an invaded natural area, and consider the effect of the manual extirpation of winter nests on this nests dynamics, on the abundance of their individuals, and on the dispersal capacity of *L. humile* over the long term.

We hope that the information provided in **CHAPTERS 1, 2,** and **3** on ecological colonization and biological strategies will help us to better understand and thus better manage the Argentine ant's expansion in invaded natural areas.

Finally, in **CHAPTER 1** we observe that in studies conducted, *P. pygmaea*–a submissive ant species which coexists with the Argentine ant in invaded areas–seemed to occupy the latter´s abandoned winter nests in spring-summer. As there are similarities in colony structure and the continuous movement of both species' nests, we believe that like the Argentine ant, *P. pygmaea* probably makes little effort to build up its nests (Newell & Barber 1913, Markin 1970, Heller 2004, Heller & Gordon 2006), leaving it to take advantage of the abandoned Argentine ant winter nests in spring-summer, and vice versa. Taking this into account, and bearing in mind that little is known about the exchange of nests between *P. pygmaea* and *L. humile*, we conducted a study on nest exchange between these two species, which we outline in **CHAPTER 4**. The purpose of this study was to identify the physical and environmental factors that promote this exchange of nesting sites between the Argentine ant and *P. pygmaea,* and ascertain whether the latter could take advantage in terms of resource competition from the invasion. To this end, for both species we examined the dynamic populations of nests in terms of nest density, worker abundance, and nest exchange. We also characterized the abiotic components (physical characteristics, temperature, and water availability) of nests for both.

STUDY AREAS

1. Gavarres and Cadiretes massifs, NE of the Iberian Peninsula, Spain.

The study area of the invaded zone is located on the northern boundary of the Catalan Coastal Range. Although this area has a strong human presence and is located near a substantially altered coastline, it offers an extensive forest landscape and has been included in the Plan of Areas of Natural Interest (PEIN in Catalan) since 1992.

We conducted the field sampling in three areas: one located at the southern edge of the Gavarres massif, in the area of Santa Cristina d'Aro (CA, 41º 48' 51.71''N; 3º 01' 50.57''E) and two in the Cadiretes massif, in the areas of Pedralta (PD, 41º 47' 31.53'' N; 2º 58' 52.79''E) and Puntabrava (PB, 41º46'13.51"N, 3º00'17.93"E) **(Figure 7)**. The three areas were at least 7 km apart from each other, with all three displaying similar physical, environmental, and ecological characteristics in both invaded and non-invaded zones.

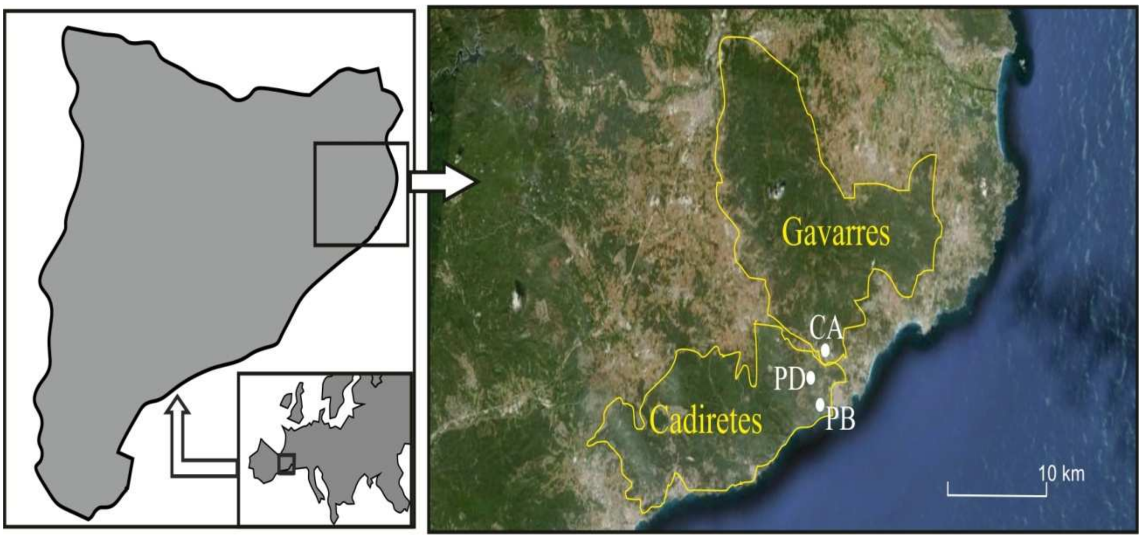


Figure 7. Location of the three study areas (CA: Santa Cristina d'Aro, PD: Pedralta and PB: Puntabrava) in the Gavarres and Cadiretes massifs, Spain. (source: Google^{TM} earth 2012)

These areas have a typical Mediterranean coastal-type climate, characterized by the coincidence of the warmest period of the year with the driest and mild winters. The mean annual temperature is 15.5ºC; with a maximum mean temperature in July and August of 20.5ºC and a minimum mean temperature in January of 10ºC. The rainfall is regular in spring and autumn, with a mean annual of 690 mm (source: Database of Automatic Meteorological Stations (EMA in Catalan) of the Catalan Government, http://www.meteo.cat/).

The soil in the study areas and in most of these two massifs is silicic in nature, which has allowed the cork oak as to establish a dominant community (*Quercetum ilicis galloprovinciale suberetosum*) **(Figure 8)**. However, as a result of great anthropogenic pressure the original cork oak forests have been reduced in this area, and there are currently many secondary cork oak forests (*Quercus suber* L.) accompanied by the presence of pines (*Pinus pinea* L.). The undergrowth consists mainly of several types of shrub, such as *Erica sp.* pl., *Cistus sp.* pl. or *Arbutus unedo* L., forming the *Cista-Sarothamnetum catalaunici* subassociation.

Figure 8. Natural area of the open cork oak secondary forest. (picture: F. Fatú)

2. Reserva Natural Otamendi (RNO), Buenos Aires, Argentina.

The study area of the native zone is located in the northeast of the province of Buenos Aires, near the Paraná River, 7 km from the city of Campana and approximately 50 km from Buenos Aires C.F. (34°14′03″S 58°53′10″W) **(Figure 9).**

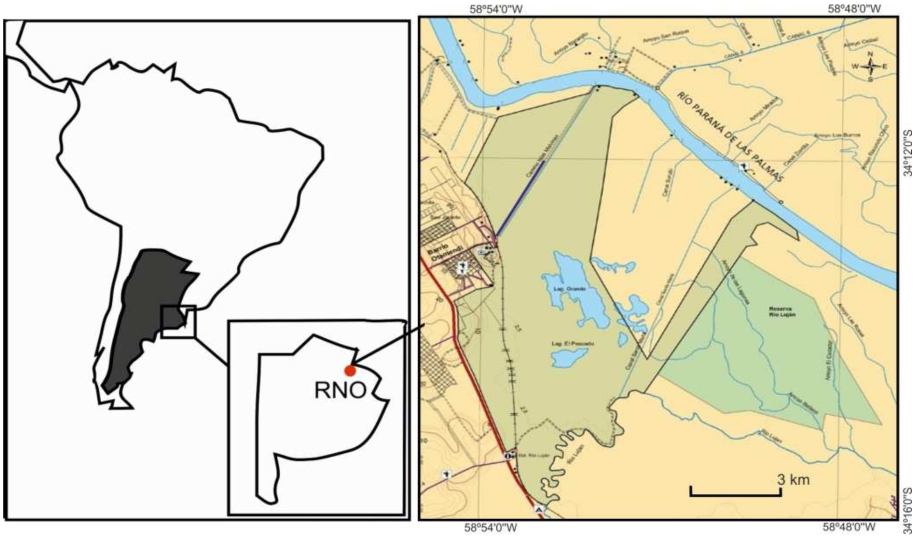


Figure 9. Native studied area in the northeast of Buenos Aires, Argentina. Figure on the left represents the geographical province of Buenos Aires; the red circle indicates the location of the Natural Reserve of Otamendi (RNO in Spanish). Figure on the right represents the RNO (source: http://www.ambiente.gov.ar/?idarticulo=5345); dark blue line indicates the 2 km section of the area sampled on the Islas Malvinas road.

We conducted the field sampling in Pampean wetlands along 2 km of the Islas Malvinas road, in an approximately 4 m wide strip of vegetation between the road and the two canals running parallel to it. This path joins the railway station Ingeniero R. Otamendi to the Paraná River. We chose the RNO because several authors have confirmed the presence of native *L. humile* colonies in this area (Wild 2004, Pedersen et al. 2006, Vogel et al. 2009).

The climate is temperate, with a mean annual temperature of 16.3ºC, a maximum mean temperature of 22 to 25ºC in summer (December-February) and a minimum mean temperature of 7 to 10ºC in winter (June-August). The rainfall is regularly distributed throughout the year, although it is more intense during the warmer season, with a mean annual of 1021 mm (source: Natural Parks Administration (PNA in Spanish), Department of Environment, Government of de Argentina).

Pampean wetlands are subject to continuous flooding as they occur over alluvial lime soils. The presence of an almost superficial water table that produces a slow permeability, together with the high annual rainfall, the Paraná River, and canal overflows, favours rain accumulation (Chichizola 1993). Due to the constant human perturbation of this specific area along the road, we found a mosaic of vegetation typical of secondary hackberry forests, riverside forests and Pampean wetlands **(Figure 10)**. Thus, the vegetation present in this area is constituted by *Celtis tala* (Gillet ex Planch), *Populus nigra* L., and various Poaceae species associated with water, such as *Deyeuxia viridiflavescens* (Poir.) Kunth (Goveto et al. 2008).

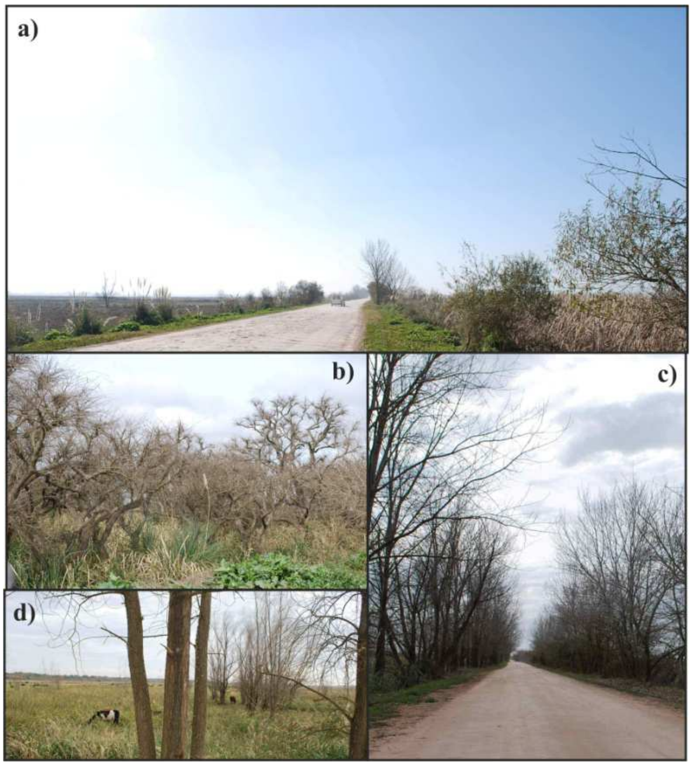


Figure 10. (a) General view of the Malvinas road with the different ecotypes: (b) secondary hackberry forest, (c) spots of riverside forests and (d) Pampean wetlands. (picture: F. Fatú)

CHAPTER 1. Where to move when it gets cold: winter nesting sites attractive to the Argentine ant (Hymenoptera: Formicidae) in their introduced and native ranges

(picture: Alice Abela)

Where to move when it gets cold: winter nesting sites attractive to the Argentine ant (Hymenoptera: Formicidae) in their introduced and native ranges

1. 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 et al. 1998, 2001, Roura-Pascual & al. 2011). Its aggressive behaviour (Carpintero & Reyes 2008) and its numerical dominance has a negative impact on native ant species, arthropod communities (Human & Gordon 1996, Holway 1998, Suarez et al. 1998, Oliveras et al. 2005), ant-vertebrate interactions (Suarez et al. 2000, Estany-Tigerström et al. 2010), and ant-plant relationships (Bond & Slingsby 1984, Visser et al. 1996, Gómez & Oliveras 2003, Gómez et al. 2003, Blancafort & Gómez 2005, Rodríguez-Cabal et al. 2009), thereby causing a drastic decrease in the biodiversity of the invaded areas.

The Argentine ant is heavily influenced by temperature and water availability. These two factors, along with others (e.g., surrounding vegetation, available food sources), are the keys to its establishment in new areas (Roura-Pascual et al. 2004, 2006, Jumbam et al. 2008). Temperature has a strong influence on some of the species' reproductive traits, such as the queen's oviposition rate (Benois 1973, Abril et al. 2008a), brood development rate (Newell & Barber 1913, Benois 1973, Hartley & Lester 2003, Abril et al. 2008a), and foraging effectiveness (Markin 1970, Human & Gordon 1999, Witt & Giliomee 1999, Holway et al. 2002a, Abril et al. 2007, Jumbam et al. 2008). Water availability determines the abundance and distribution of the Argentine ant in Mediterranean-type systems (Human & Gordon 1999, Suarez et al. 2001, Holway et al.

2002a, Jumbam et 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 et 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 et al. 2002a, Abril et al. 2007) and why low soil moisture limits its expansion (Holway et al. 2002a, Menke & Holway 2006, Menke et al. 2007).

In invaded natural areas, the Argentine ant changes its nesting preferences seasonally according to food availability (Heller et al. 2006) and its physiological temperature and humidity requirements (Newell & Barber 1913, Markin 1970, Benois 1973, Heller & Gordon 2006, Abril et 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 et al. 2008b). Ants usually show preferences for nesting sites (Fernández-Escudero et 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 et al. 1999, Thomas 2002, Robinson 2008, McCaffrey & Galen 2011). The Argentine ant is also known to build its nests generally under rocks (Cole et al. 1992, Ingram 2002a) 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 (Silverman & Brightwell 2008). 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 et 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 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 is therefore to: (1) determine the distinct supercolonies present in the native range, as well as their number, distribution and density of nests; (2) determine the nesting preferences of this invasive species in winter, in both introduced and native ranges; and (3) discover whether the winter nesting behavior of the Argentine ant is intrinsic or was acquired when it became an invasive species.

This information on ecological colonisation strategies in native and introduced ranges could help us to better understand, and thus, to better manage its expansion in natural invaded areas.

2. MATERIAL AND METHODS

2.1 Study areas

2.1.1 Introduced range

The study in the introduced range 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 north east (NE) of the Iberian Peninsula. This region has a Mediterranean climate with 690 mm of mean annual rainfall and a mean temperature of 15.5ºC (Database of Automatic Meteorological Stations (EMA) of the Catalan Government) **(Figure 1).** During the two years of the survey, winters 2008/2009 and 2009/2010 presented the following temperature and rainfall values: Maximum temperature: 23.3ºC / 19.4ºC, Minimum temperature: -1.5ºC / -3.6ºC and Rainfall: 72.4 mm / 55.10 mm, respectively (Database of Automatic Meteorological Stations (EMA) of the Catalan Government).

Winter nests were sought following the main human-path of each zone sampled. We entered on both sides of the path to where the forest was accessible and until we found all needed nests for each sampling (i.e. 90 winter nests). Each of the winter nests were searched lifting every single rock, branch or piece of debris, and gently moving the leaf litter with the help of a shovel at the bases of trees. Previous observations in the study area confirmed that the Argentine ant nests were not greatly disturbed by this sampling method, and that nests remained in place after several weeks.

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.

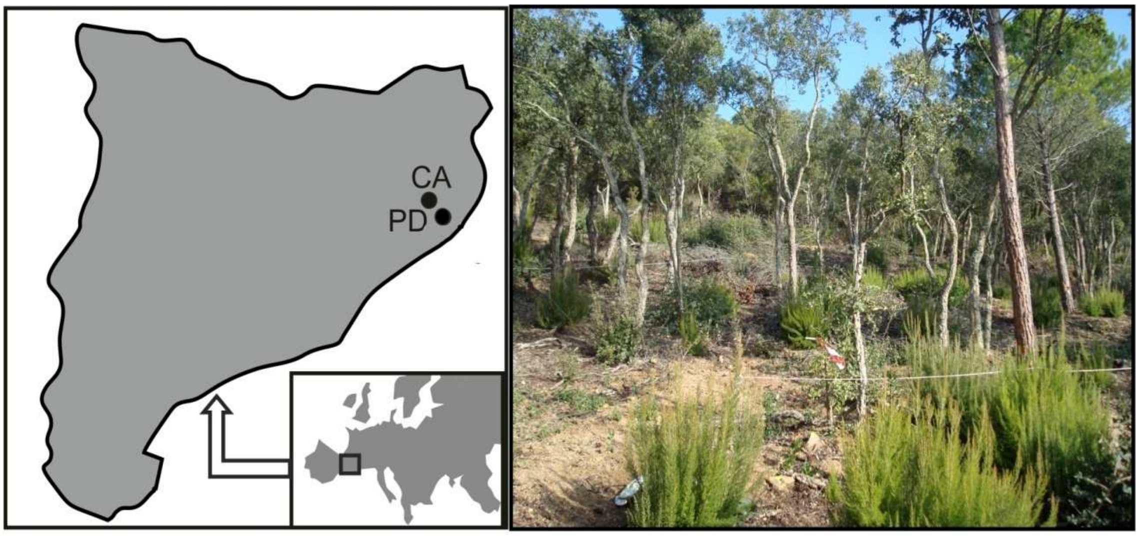


Figure 1. Introduced studied area in the NE of the Iberian Peninsula. Black circles indicate each locality sampled: CA (Santa Cristina d’Aro) and PD (Pedralta). Picture on the right represents the natural area sampled of the open cork oak secondary forest. (picture: F.Fatú)

2.1.2 Native range

The study in the native range was carried out in the austral winter of 2011 (July and August) in the Natural Reserve of Otamendi (34°14′03″S 58°53′10″O), approximately 50 km north of Buenos Aires, Argentina. It was conducted in Pampean wetlands along the first 2 km of the Islas Malvinas road that joins the railway station Ingeniero R. Otamendi with the Paraná River (Wild 2004, Pedersen et al. 2006, Vogel et al. 2009). This region also has a Mediterranean climate with a mean annual rainfall of 1021 mm and a mean temperature of 16.3ºC **(Figure 2).**

Winter nests were sought on both sides of the road, in approximately 4 m wide strips of vegetation between the road and the canals running parallel to it **(Figure 2).**

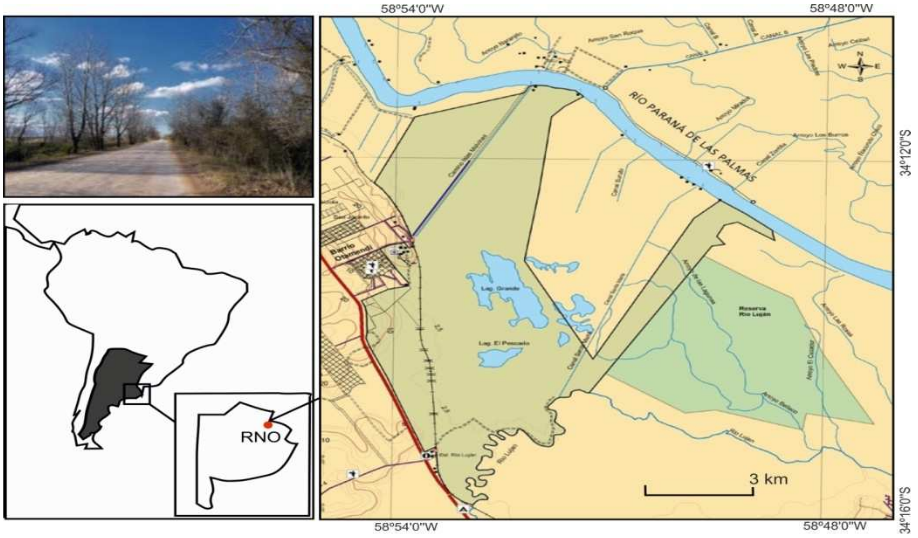


Figure 2. Native studied area in the north of Buenos Aires, Argentina. Figure on the bottom left represents the geographical province of Buenos Aires; the red circle indicates the location of the Natural Reserve of Otamendi (RNO in Spanish). Figure on the right represents the RNO (source: http://www.ambiente.gov.ar/?idarticulo=5345); dark blue line indicates the 2 km section of the area sampled on the Islas Malvinas road and picture (F. Fatú) on the top left represents the native area sampled of these 2 km section.

2.2 Supercolonies in the native range: number, distribution and density of nests

In order to characterize the Argentine ant population in the RNO, we followed the same procedure as Vogel et al. (2009). We conducted standard aggression tests between pair of workers from adjacent nests (Holway et al. 1998, Giraud et al. 2002) to determine the number of distinct supercolonies present along the first 2 km of the Islas Malvinas road, from the railway station to the Paraná River. We considered a "supercolony" to be each group of nests linked with a complete lack of aggression between each other, but with a very high aggression among supercolonies (Holway et al. 1998, Giraud et al. 2002, Vogel et al. 2009). For this purpose, we randomly selected a single worker from each adjacent nest and placed them together in a neutral arena (2.5 cm diameter vial with fluon-coated sides). The tests began with the first interaction and continued for five minutes. The level of aggression was scored as Vogel et al. (2009): 0

= ignore, physical contact in which neither ant showed any interest; 1 = antennation, repeated tapping of the antennae somewhere on the other ant; 2 = avoidance, one or both ants retreating in opposite directions after contact; 3 = dorsal flexion, gaster raised to vertical position as escalation to chemical defense; 4 = aggression, biting, pulling of extremities and/or head, or deposition of venom; and 5 = fight, prolonged aggression, often involving locking the mandibles onto a body part of the other ant or carrying it. Levels 0 to 2 are referred to as non-aggressive behavior and levels 3 to 5 are referred to as aggressive behavior. Different workers were used in the three trials conducted for each pair of adjacent nests.

Colony distribution and their density index were assessed recording the presences or absences of nests each meter along the sampled transect (Vogel et al. 2009).

2.3 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) in the introduced range and a total of 44 nests in the native range. We registered the following variables for each of them in both native and introduced ranges: canopy cover above the nest (%), orientation (i.e., the main direction towards which the nest was facing taking into account the direction of the nest slope), and distance to the nearest nest (m) and to the nearest tree (m). We recorded the location of each nest using a Garmin eTrex Legend® HCx GPS with an accuracy of 3 m, as well as orientation and distance to the nearest nest (calculating the Euclidean distances with Mapsource_v6.13.7 Extreme GPS software). 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 (%).

In addition, in the introduced range, we also reported the distance to the nearest human-made path (i.e. walking track (m)) using the same method as for distance to the nearest tree. When the nest was located under a rock, we also measured its surface in cm^2 (maximum x minimum diameter), and its colour. The colour of the rock was categorised as follows: (1) light-coloured and (2) dark-coloured or colonised by lichens or mosses, taking a colour standard as shown in **Picture 1**.

Picture 1. Photographs showing the gradual scale of rock colour. (picture: M. Diaz)

We also sampled 90 control points (50 in CA and 40 in PD forests) and 44 control points (in RNO), free of Argentine ant nests. Each of these control points was 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.

2.4 Temperature and water content

We measured the soil temperature (ºC) and soil volumetric water content (VWC, %) of winter nests and their respective control points in both introduced and native ranges. 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 winter nests/control points we also measured the soil VWC three times on the day of 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.

2.5 Monitoring and nest site fidelity in the introduced range

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

2.6 Data analysis

We conducted descriptive analyses of the nests to gain an overall view of the characteristics of each native supercolony (number, distribution and nest density), and of the physical characteristics of winter nests used by Argentine ants in each range. We compared the canopy cover, temperature, and water content of nests and control points in both ranges using generalised linear mixed models (GLMMs). In the case of canopy and VWC we used a Poisson error distribution and a log link function, and in the case of temperature a Gaussian error distribution and an identity link function. Sample identity was used as a random factor and sample type (nest or control) as a fixed factor.

To assess whether the physical and environmental characteristics of winter nests are intrinsic or have changed as the ants have become an invasive species, we performed generalised linear models (GLMs). We compared the distance to the nearest tree, distance to the nearest nest, and temperatures using a Gaussian error distribution with an identity link function, and for orientation we ran a multinomial logistic regression (MLR).

In the introduced range, we also 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 colour. For comparisons we used GLMMs with a binomial error distribution and a log link function, using locality as a random factor and variables (1 to 6) as fixed factors. To evaluate the environmental factors that may influence the occupation of winter nests in the introduced range, we performed 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 both the introduced range (winter 2009/2010) and the native range (austral winter 2011).

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 from the introduced range. Temperature was log-transformed to achieve normality in lineal regressions from the native range.

3. RESULTS

3.1 Supercolonies in the native range: number, distribution and density of nests

We found six supercolonies along the first 2 km sampled on both sides of the road from the railway station to the Paraná River **(Figure 3)**. The mean level of aggression value for workers from the same supercolony was very low, 1 ± 0.07 (SE), compared with the high level of aggression between pairs of supercolonies, 4.31 ± 0.24 (SE). The characteristics of each of the six supercolonies are given in **Figure 3** and **Table 1**.

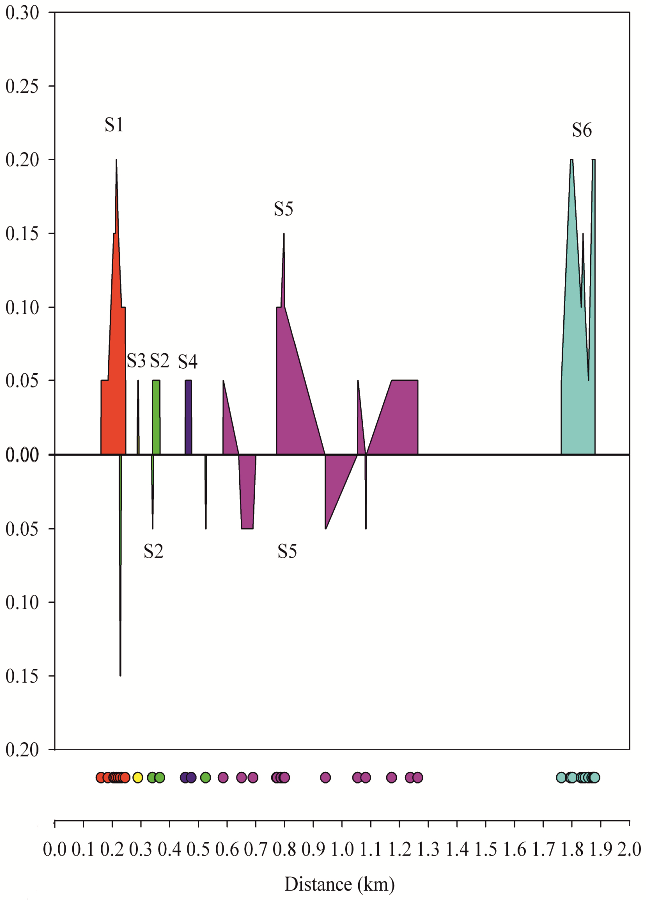
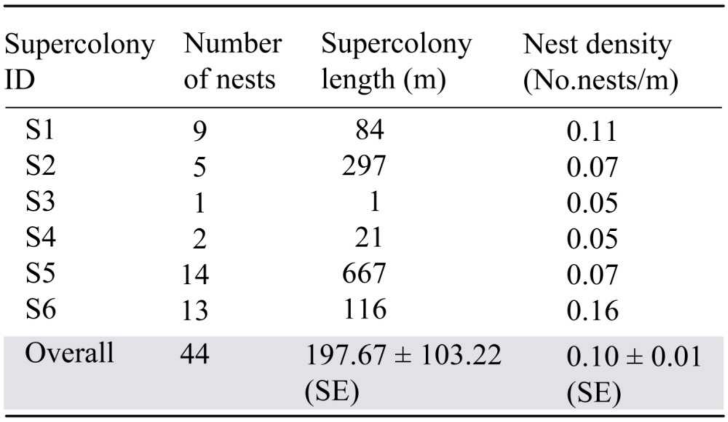


Figure 3. Localisation of the Argentine ant nests sampled in the native range and nest density along the 2 km transect. The circles and their colour indicate nest location and their assignment to supercolonies (S1, S2, S3, S4, S5 and S6) on the basis of behavioural data. Data at the top of the figure are nests on the right-hand side of the Islas Malvinas road and data at the bottom of the figure are nests on the left-hand side.

The mean length of each supercolony was very variable, 197.67 ± 103.22 m (SE), from 1 m for the smallest supercolony and 667 m for the largest one **(Table 1)**. The distance between supercolonies was also variable with a mean 112.57 ± 65.85 m (SE) and a range from 16 to 506 m. Mean nest density was 0.10 ± 0.01 nest/m (SE) **(Figure 3)**.

Table 1. Characteristics of the Argentine ant supercolonies at the Natural Reserve of Otamendi. Number of nests refers to the number of adjacent nests of each supercolony sampled for aggression tests.



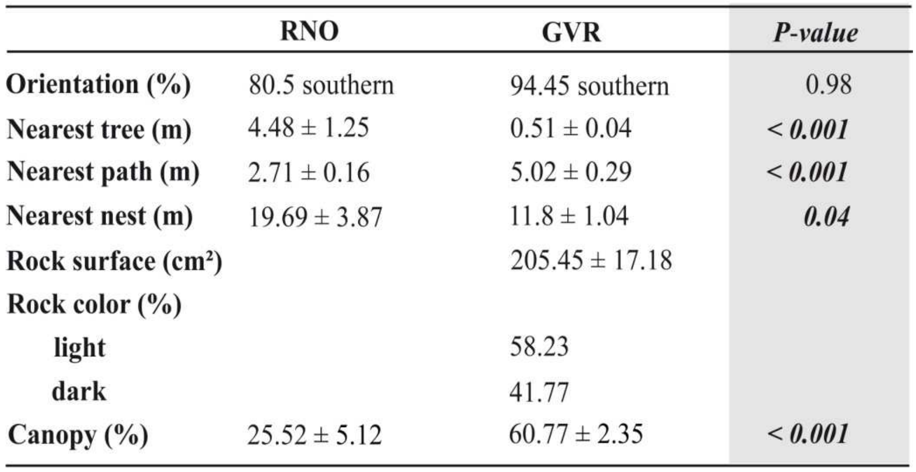
Supercolony ID	Number of nests	Supercolony length (m)	Nest density (No.nests/m)
S1	9	84	0.11
S2	5	297	0.07
S3	1	1	0.05
S4	2	21	0.05
S5	14	667	0.07
S6	13	116	0.16
Overall	44	197.67 ± 103.22 (SE)	0.10 ± 0.01 (SE)

Comparisons of physical factors between the six supercolonies found in the native range only were significant in terms of distance to the nearest nest (GLM: $F = 3.27$, $P = 0.02$), indicating that larger supercolonies presented long distances to the nearest nest ($r^2 = 0.16$, $P = 0.015$). In regard to temperature and VWC all six supercolonies were the same (GLM, temperature: $F = 2.3$, $P = 0.08$; VWC: $P\ (>|\chi^2|) = 0.10$).

3.2 Physical factors

We compared the winter nests in the native range with those in the introduced one. We found differences in all variables except for orientation. Comparisons between physical factors in native and introduced ranges are given in **Table 2**.

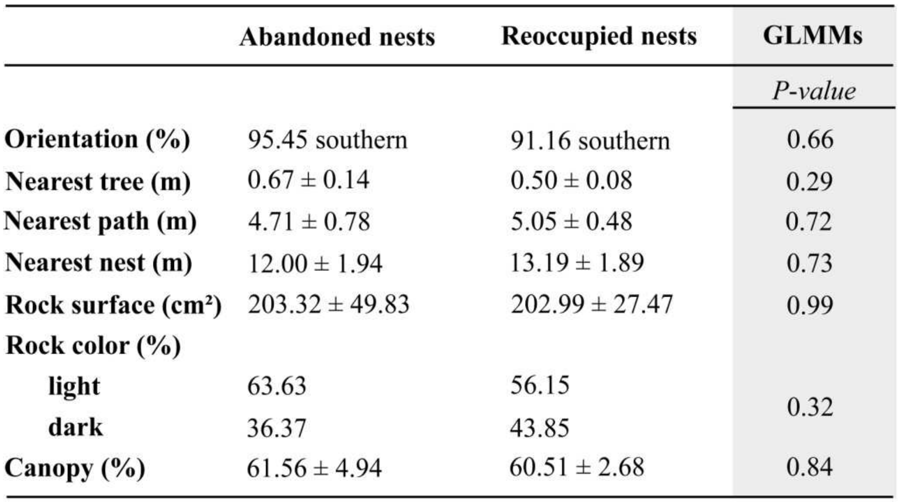
Table 2. Characteristics of the winter nests at the Natural Reserve of Otamendi (RNO) and the Gavarres massif (GVR) (mean ± SE). *P-value* in bold italic is a significant value ($P < 0.05$).



	RNO	**GVR**	***P-value***
Orientation (%)	80.5 southern	94.45 southern	0.98
Nearest tree (m)	4.48 ± 1.25	0.51 ± 0.04	***< 0.001***
Nearest path (m)	2.71 ± 0.16	5.02 ± 0.29	***< 0.001***
Nearest nest (m)	19.69 ± 3.87	11.8 ± 1.04	***0.04***
Rock surface (cm²)		205.45 ± 17.18	
Rock color (%)			
light		58.23	
dark		41.77	
Canopy (%)	25.52 ± 5.12	60.77 ± 2.35	***< 0.001***

On the one hand, winter nests in the introduced range faced mainly a southern direction. A total of 87.78% (79/90) of these nests were located under rocks, 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 indicated 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 or another nest. There was a great variation between these two latter distances: from 0.1 to 12 m and from 2 to 39 m respectively, and the mean rock surface: from 12 to 1176 cm^2. Mean canopy cover (%) was significantly higher in winter nests (60.77 ± 2.35%) than in control points (51.25 ± 2.71%) (GLMM: $P\ (>|\chi^2|) < 0.001$) **(Figure 4).** GLMMs performed between abandoned and reoccupied winters nests in 2008/2009 were not significant for all of these variables **(Table 3)**.

Table 3. Physical characteristics of the abandoned and reoccupied winter nests in 2008/2009 (mean ± SE). GLMMs (Generalised Linear Mixed Models). *P-value* in bold italic is a significant value ($P < 0.05$).



	Abandoned nests	**Reoccupied nests**	**GLMMs**
			P-value
Orientation (%)	95.45 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 (cm²)	203.32 ± 49.83	202.99 ± 27.47	0.99
Rock color (%)			
light	63.63	56.15	0.32
dark	36.37	43.85	
Canopy (%)	61.56 ± 4.94	60.51 ± 2.68	0.84

On the other hand, winter nests in the native range faced also a southern direction in most of the cases. The mean distance to the nearest nest, likewise in the introduced range presented a wide ranger values: from 1.3 to 59 m. This variation may be due to the ecological context of the native study area, a Pampean wetland with patches of riverside trees along both sides of the road. Mean canopy cover was also higher at winter nests (25.52 ± 5.12%) than at control points (16.63 ± 3.86%) (GLMM: $P\ (>|\chi^2|) < 0.001$) **(Figure 4)**.

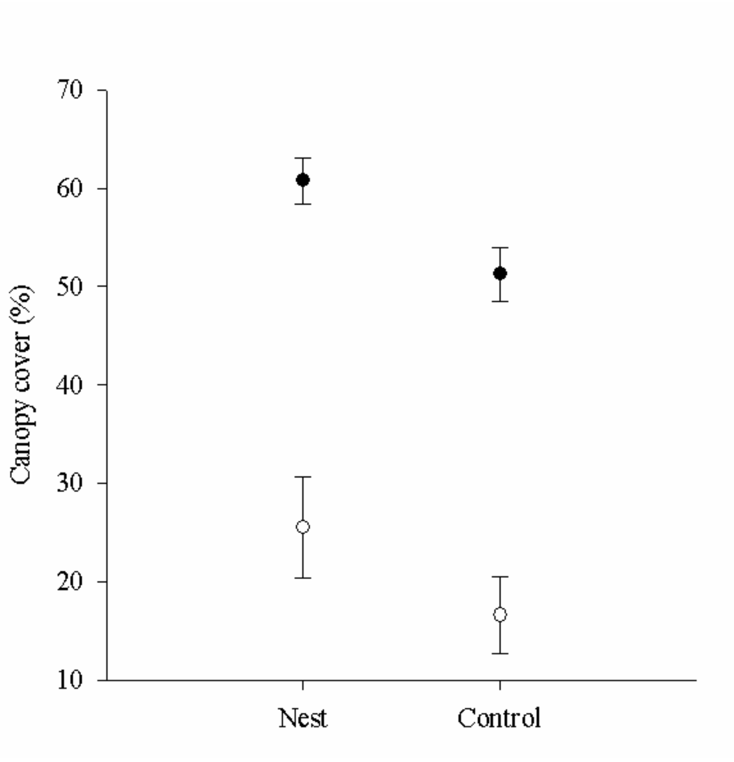
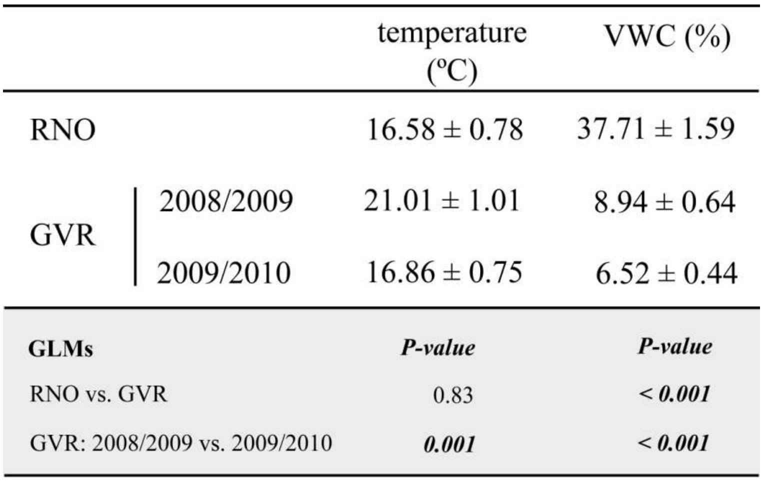


Figure 4. Canopy cover (%) (mean ± SE) of winter nests and control points at the Gavarres massif (filled dots, n=90) and the Natural Reserve of Otamendi (open dots, n=44).

3.3 Temperature and water content

We compared temperature and VWC between the winter nests in the native range with those found in the introduced one. We found differences for VWC, but not for temperature **(Table 4)**.

Table 4. Winter nests temperature (mean ± SE) (ºC) and VWC (%) (mean ± SE) at the Natural Reserve of Otamendi (RNO) and the Gavarres massif (GVR), winters 2008/2009 and 2009/2010. GLMs (Generalised Linear Models). *P-value* in bold italic is a significant value ($P < 0.05$).



		temperature (ºC)	VWC (%)
RNO		16.58 ± 0.78	37.71 ± 1.59
GVR	2008/2009	21.01 ± 1.01	8.94 ± 0.64
	2009/2010	16.86 ± 0.75	6.52 ± 0.44
GLMs		***P-value***	***P-value***
RNO vs. GVR		0.83	***< 0.001***
GVR: 2008/2009 vs. 2009/2010		***0.001***	***< 0.001***

In the introduced range, 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 **(Table 4)**.

Mean soil temperatures ± SE of winter nests (16.86 ± 0.75ºC) and control points (15.8 ± 0.69ºC) in winter 2009/2010 were not significantly different from each other (GLMM: $F = 3.37$, $P = 0.07$). However, the mean soil water content was higher at control points (7.54 ± 0.52%) than at winter nests (6.52 ± 0.44%) (GLMM: $P\ (>|\chi^2|) = 0.01$) **(Figure 5).** The range of temperatures from winter nests under light and dark-coloured rocks was 2.46 to 43.42ºC (16.71 ± 0.83ºC) and -1.06 to 50.11ºC (18.71 ± 1.46ºC), respectively. A total of 26.67% (8/30) of dark-coloured rocks reached superficial nest soil temperatures up to 40 to 45ºC, while light rocks only once reached superficial nest soil temperatures up to 40ºC **(Figure 6)**.

In the native range, mean soil temperatures ± SE of winter nests (16.58 ± 0.78ºC) and control points (16.59 ± 0.77ºC) did not differ between each other (GLMM: F =

0.0005, *P* = 0.98). But the mean soil water content was higher in control points (48.87 ± 2.12%) than in winter nests (37.71 ± 1.59%) (GLMM: *P* (>|χ^2|) < 0.001) **(Figure 5)**.

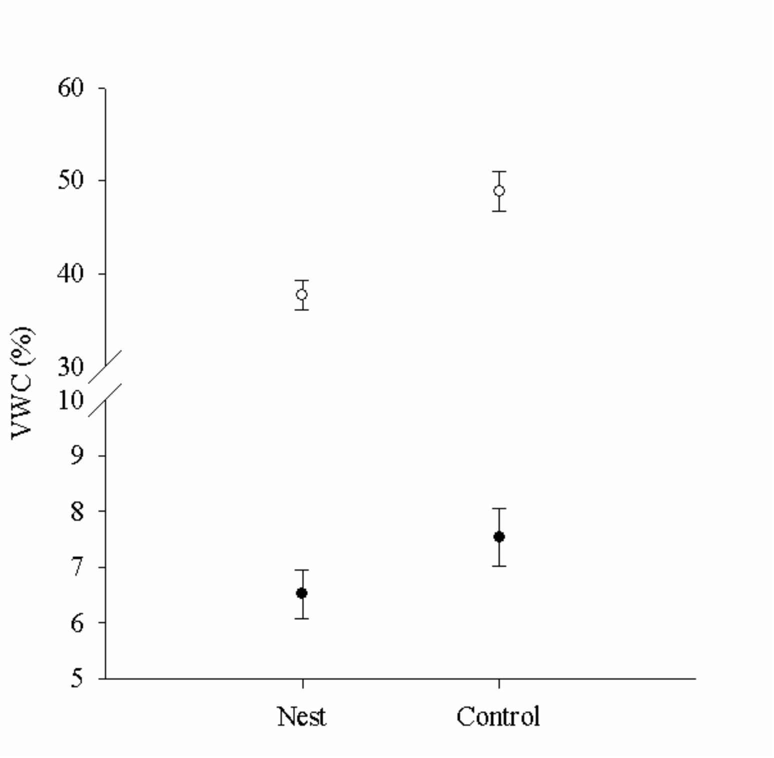


Figure 5. Volumetric water content (VWC, %) (mean ± SE) of winter nests and control points at the Gavarres massif (filled dots, n=90) and the Natural Reserve of Otamendi (open dots, n=44).

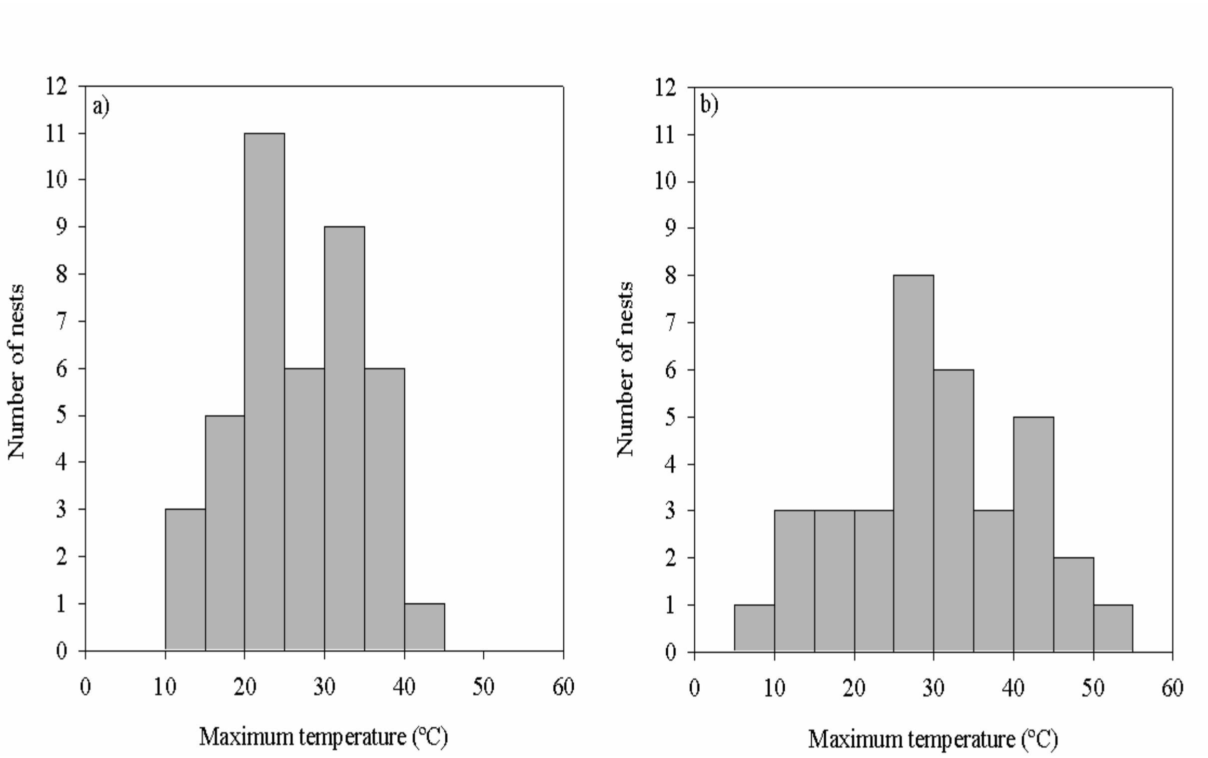


Figure 6. Histograms of the maximum temperature (ºC) reached under (a) light-coloured rocks (n=46) and (b) dark-coloured rocks (n=33) in the introduced range.

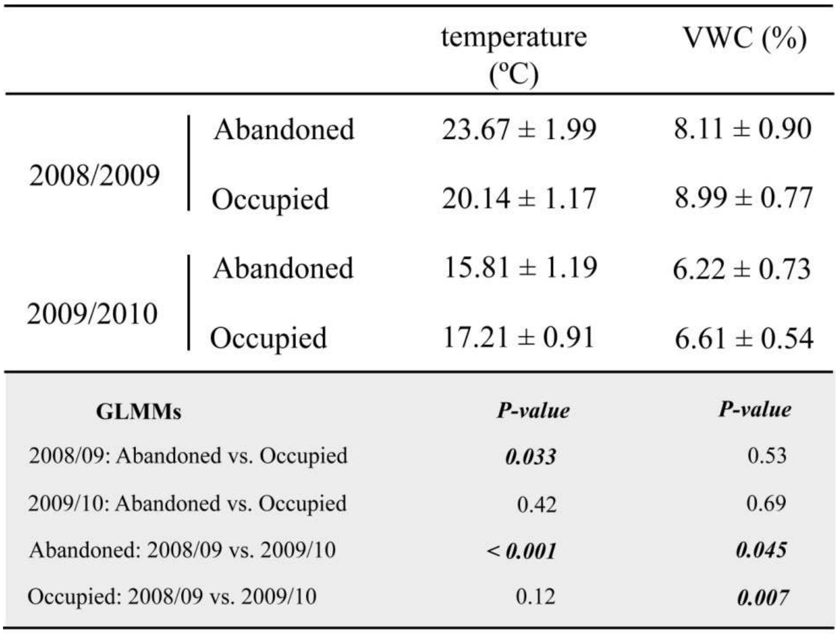
Linear regressions showed a slight relationship between winter nest soil water content and nest temperature in both ranges (introduced range: r^2 = 0.11, P < 0.01; native range: r^2 = 0.077, P = 0.021)**.** Moreover, in the introduced range winter nest water content also depended on the canopy cover above them (r^2 = 0.07, P < 0.001).

3.4 Monitoring and nest site fidelity in the introduced range

Colonies remained the whole year in the same nest in 15.6% (14/90) of the cases. During 2009, the degree of nest site fidelity decreased gradually throughout the year (51.1% in spring, 38.9% in summer and 27.8% in autumn). In winter 2009/2010, the Argentine ant returned to most of the monitored nests, concretely 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 that the reoccupation of nests is related to their temperature and soil moisture **(Table 5)**. 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. Additionally, the mean temperature of nests occupied in winter 2009/2010 and those nests not abandoned in 2008/2009 remained the same in both years **(Figure 7a)**. 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) winter **(Figure 7b)**.

Table 5. Temperature (ºC) (mean ± SE) and VWC (%) (mean ± SE) of the abandoned and occupied nests in winters 2008/2009 and 2009/2010. GLMMs (Generalised Linear Mixed Models) *P-value* in bold italic is a significant value (*P* <0.05).



		temperature (ºC)	VWC (%)
2008/2009	Abandoned	23.67 ± 1.99	8.11 ± 0.90
	Occupied	20.14 ± 1.17	8.99 ± 0.77
2009/2010	Abandoned	15.81 ± 1.19	6.22 ± 0.73
	Occupied	17.21 ± 0.91	6.61 ± 0.54
GLMMs		***P-value***	***P-value***
2008/09: Abandoned vs. Occupied		***0.033***	0.53
2009/10: Abandoned vs. Occupied		0.42	0.69
Abandoned: 2008/09 vs. 2009/10		***< 0.001***	***0.045***
Occupied: 2008/09 vs. 2009/10		0.12	***0.007***

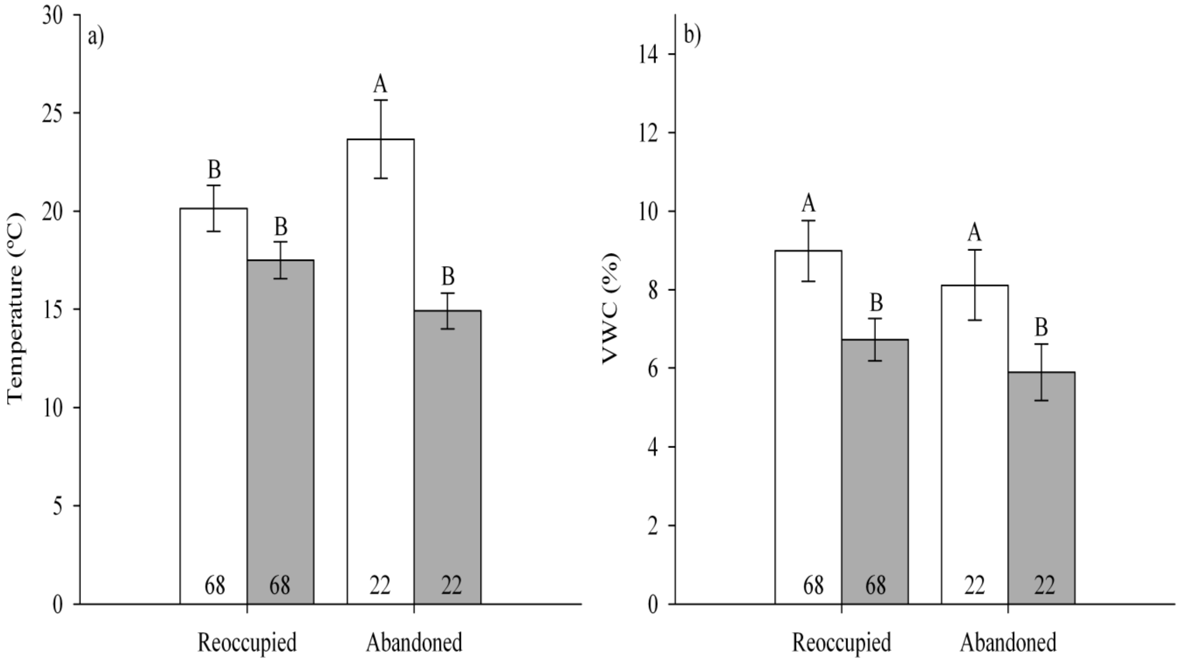


Figure 7. (a) Temperature (ºC) (mean ± SE) and (b) VWC (%) (mean ± SE) of abandoned and reoccupied winter nests in 2008/2009 (open bars) and 2009/2010 (grey bars). Sample sizes are shown inside bars. Letters indicate significant differences or similarities between abandoned or reoccupied nests among years.

4. DISCUSSION

Vogel et al. (2009) suggested that native supercolonies could be considered to be homologous to the introduced supercolonies, and that the only differences between zones are their respective sizes. Furthermore, it seems that native supercolonies are smaller because they are additionally submitted to the pressure from other closer supercolonies, native competitors and parasites (Vogel et al. 2009). These ecological features do not allow supercolonies in the native range to grow and expand as much as they do in introduced areas where this ant species is invasive (Suarez et al. 2001).

On the other hand, Heller (2004) suggested that native supercolonies are able to grow and expand their spatial size by several meters every year. However, we observed that the spatial distribution of supercolonies in austral winter 2011 in the RNO was similar that which Vogel et al. (2009) found in their study. Native supercolonies in the firstly 2 km of the Islas Malvinas road were almost the same with regards to number, length and distance between each other as those found by Vogel et al. (2009) in this area. On the other hand, different results in the mean nest density of native supercolonies within studies could be a consequence of the different sampling period chosen. While we assessed nests in austral winter (July-August), they took the samples during austral spring (October-November). In fact, this could be the reason why mean nest density found by Vogel et al. (2009) were more than doubled those we found in winter, suggesting that they found more but probably smaller nests due to the fission period of the year. Taking this into account, it could be suggested that as in the introduced range, it seems that nests within supercolonies in the RNO follow the typical cycle of fusion in winter and fission in spring (Heller & Gordon 2006). In fact, if we consider native supercolonies to be homologous to introduced supercolonies (Vogel et al. 2009), in winter, nests will be aggregated and the spatial range of these supercolonies will be retracted (Enríquez ML, Abril S, Diaz M and Gómez C, unpubl.), thus becoming further from each other. Moreover, later in spring native supercolonies may expand their boundaries and the distance between their territory borders could be reduced. This could well be likely the reason why we found the same length and almost the same distance between supercolonies but with a lower nest density in winter two years later (austral winter 2011).

On the other hand, physical and environmental characteristics were similar for each supercolony and for each studied area (native and introduced), as their individual winter nests were located in sites of the same kind.

Thus, the Argentine ant prefers to nest in winter facing south and in places near some plant structure, which provides them a close food resource and a canopy cover above the site. Additionally, it also prefers to nest in areas where temperature and soil moisture are moderate, rather than in areas with extreme climate conditions (Menke et al. 2007). Moreover, in invaded areas winter nests also were found mostly under rocks. Its nesting behaviour (Newell & Barber 1913, Markin 1970, Heller 2004, Heller & Gordon 2006) and its physiological characteristics (Holway 1998a) 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 help to maintain optimal conditions inside the nest for winter activity. They mainly include soil moisture, temperature, factors related to the vegetation around the nest, nest orientation in both ranges, as well as rocks in the introduced one.

However, Roura-Pascual et 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 on a global scale. Moreover, Roura-Pascual et al. (2004) also predicted an expansion of ant species to higher latitude areas in the future. Against this background, we may have to take into account the data found on the Argentine ant abiotic preferences for nesting to predict which zones, where this ant species is not yet present because low temperatures, may be susceptible to invasion in the future due to increasing temperatures with climate change. We therefore expect that Argentine ant preferred nesting locations will be the same in these future potential invaded zones as those found in our study area.

Winter nests were located in places with a mean daily nest soil temperature of 15.81 to 17.37ºC during mid-winter in Otamendi. These temperatures do not differ from those found in the natural invaded areas of the Gavarres and Cadiretes massifs (16.11 to

17.61ºC), but both were slightly higher than those reported by Heller & Gordon (2006) in California (13.1 ± 0.4ºC SD). However, the general trend was similar, with the Argentine ants more likely to nest in moderately warm sites, which suggests that nest soil temperature influences nest location. Additionally, these results also fit within the range of temperatures found by Brightwell et al. (2010) for foraging activity during winter in northern California. This suggests that the mid-winter nest soil temperatures found in both ranges could also permit foraging activity during this winter and thus, they would explain the one observed during this time by Abril et 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 time maintaining it at low levels and avoiding desiccation. Nest soil temperature is in turn controlled by other physical factors of the nesting site, such as orientation in both ranges (native and introduced), and rocks in the introduced one. Canopy cover above winter nests may be related to the distance these nests are from plant structures, which at the same time provide them with a food resource near the nesting site. This would explain why the Argentine ant prefers to establish its nests closer to trees and shrubs in both ranges, as Heller & Gordon (2006) found in California. Additionally, although canopy cover seems not to be a clue factor for nesting site selection, it could have a two-side role, helping to avoid extreme temperatures and also to avoid 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 seems that dominant species in Mediterranean environments usually have lower thermal tolerance (Cerdà et al. 1998, Retana & Cerdà 2000). In that sense, the Argentine ant, which is a well-known dominant species in this natural invaded areas (Holway et al.

2002a), could avoid reaching extreme temperatures inside nests locating them in sites protected by the vegetation. At the same time, the canopy cover in the introduced range could be acting as a protection against precipitation and optimizing moisture levels for the nest. In fact, this is what seemed to happen in the control points, where the soil water content was much higher than that of winter nests. Although soil moisture in the native range was also higher in control points than in winter nests, it seems that canopy cover is not as important in preventing sudden soil moisture fluctuations in this area as it is in the introduced range. This is because of the flooding dynamics in Otamendi.

Soil water content in winter nests is much lower than in control points in both ranges, and it was higher in the native range than in the introduced one. These great differences may be due to the ecological context of the two studied areas. In the native range, Pampean wetlands are subject to continuous flooding. A mean annual rainfall of 1021 mm, the Paraná River, canals floods and the presence of an almost superficial water table that produces a slow permeability, favors rain accumulation. Contrarily, in the invaded areas of Mediterranean cork oak forests the mean annual rainfall is 690 mm and the geological characteristics of the area confer a higher permeability, as well as a much lower flood risk. These soil moisture conditions need to be regulated by temperature and orientation in the native range, and by these two factors along with the help of canopy cover above winter nests in the introduced one.

Furthermore, it seems to be that orientation in both ranges and rocks in the introduced range may also help to protect the nest from these extreme temperatures, as well as capture sunlight to provide an additional heat source to regulate nest soil moisture and therefore maintain optimal environmental conditions inside winter nests. The main reason the Argentine ant nest faces 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 (< 5ºC longer than 9 days, Brightwell et al. 2010), as well as regulate high percentages of soil water content inside nests. In the native range this is an important issue, as in Otamendi the Argentine ant is submitted to continuous overflows from the Paraná River and the two canals alongside the road. In addition, this could also help to maintain some ant activity inside the nests or even to facilitate foraging when the ambient temperature is below the minimum foraging threshold for *L. humile* during winter (Abril et al. 2007, Brightwell et al. 2010). On the other hand, several authors have suggested that rocks can have thermoregulatory properties, which could influence the soil temperature around them (Hölldobler & Wilson 1990, Nobel et al. 1992), protect the colony against extreme temperatures, and provide a supplementary source of heat (Hölldobler & Wilson 1990, Tinaut et 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 et al. 1999, Fernández-Escudero & Tinaut 1999, Robinson 2008, McCaffrey & Galen 2011). Thus, we can suggest that the Argentine ant may also have preference for a specific nest site type in the introduced range, as its winter nests were built more often under rocks. Although the rock colour seems not to be a key factor in nesting site selection, there were somewhat more light-coloured than dark-coloured rocks. Light-coloured rocks probably help to regulate critically high temperatures (> 40 to 44ºC, Jumbam et al. 2008) and may prevent the heating effect that may cause the dark-coloured rocks. In fact, the elevated temperatures that some of the abandoned dark-coloured nests reached (up to 45ºC), could be a reason why those nests were left behind. This suggests that the abandonment of these nests during this period in the introduced range was due in part to these extreme temperatures, which forced colonies to relocate to other nests in order to find cooler and somewhat 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 the introduced range may take advantage of all rocks available for nesting regardless of their size, suggesting that they do not have a greater preference for any particular rock size when funding their nests. However, other ant species such as *Proformica longiseta* (Collingwood 1798) preferred larger rocks for nesting, but rock size varies because of rock size availability, taking into account the necessary range for nesting (50-250 cm^2). These results suggested that there was not a real rock selection regarding to rock dimensions, and that *P. longiseta* seemed to choose suitable rocks on the basis of their thermal properties (Tinaut et 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.

In the introduced range during the survey period, environmental changes occurred in the study region. The first winter (2008/09) was the hottest and driest in the ten last years, and the second winter (2009/10) was the coldest in the last two decades, but driest then the previous winter (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 et al. 1999). The Argentine ant is one of those ants which shifts its nesting behaviour depending on the requirements of the moment (Newell & Barber 1913, Markin 1970, Benois 1973, Heller & Gordon 2006, Heller et al. 2006, Abril et al. 2008b). The higher temperatures and dryness during the first winter could have triggered the shift of nest locations to more humid places to avoid desiccation, which could be the reason why we found higher soil moisture inside nests during in winter 2008/09. Moreover, these harsh conditions could be one of the reasons why the Argentine ant abandoned some of the winter nests of 2008/09 and looked for more suitable sites to nest the following winter. However, bimonthly monitoring confirmed that in a high percentage of cases (75.56%) the Argentine ant returns to the same winter nesting place year after year. A total of 15.6% 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 et al. (2008a). These mother nests may constitute a major 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 **CHAPTER 3**. In this section, larger nests in invaded areas remained inhabited longer, or 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 2009/10 in **CHAPTER 3** were probably mother nests.

In summary, as Vogel et al. (2009) suggested, native supercolonies could be compared with and considered to be homologous to introduced supercolonies in all but size. First, it seems that they also follow a yearly pattern of fusion-fission of nests and second, their nesting behaviour in winter is similar to that in invaded areas. The location of Argentine ant winter nests is influenced mostly by soil moisture and temperature, as well as factors regulating them. Therefore, a relative distance to the nearest tree and southern orientations in both ranges, and canopy cover and the shelter of rocks in the introduced range help to avoid high levels of soil moisture. These characteristics also protect the colony from extreme temperatures, and maintain optimal environmental conditions both for inside nests, and for colony activity during winter. Against this background, we conclude that winter nesting sites are similar in both introduced and native ranges. Differences observed in some of the factors studied here are due to the contrasting ecological contexts of the Natural Reserve of Otamendi and the Gavarres and Cadiretes Massifs. Thus, as Vogel et al. (2009) suggested, the success of the Argentine ant as an invasive species does not rely in a shift in social organisation nor on a shift in its mode of nesting associated with the introduction to new habitats. Moreover, the winter nesting behaviour of the Argentine ant in the invaded areas seems to be intrinsic and it needs only to make minor behavioural adjustments to allow it to take advantage of all the available resources in the different ecological contexts in which it is nesting.

This information helps to improve the knowledge of Argentine ant’s nesting behaviour in winter in its native and introduced ranges, as well as information about the spatial range of winter nests and native supercolonies. However, further data about queen biology and physiology in both ranges are needed to better understand the expansion, and thus, to initiate control methods based on the reduction of queens inside winter nests in invaded natural areas.

CHAPTER 2. Shifts in energy investment in queens of the Argentine ant in response to different ecological contexts

(picture: Alex Wild)

Shifts in energy investment in queens of the Argentine ant in response to different ecological contexts

1. INTRODUCTION

The Argentine ant (*Linepithema humile*, Mayr 1868) is a well-known invasive species (Lowe et al. 2000). Native to South America, it has spread worldwide through commercial activities (Suarez et al. 2001) to areas with Mediterranean-type climates associated, usually, with human-altered habitats (Suarez et al. 1998). However, its ability to invade natural areas with low levels of anthropogenic disturbance is also widely recognised (Holway 1998a, Gómez et al. 2003). In these areas, its effects on the ecosystem are extremely harmful since it negatively impacts native ant faunas (Human & Gordon 1996, 1997, Holway 1998b, Suarez et al. 2001) and causes changes to important ecological processes such as seed dispersal (Bond & Slingsby 1984, Gómez & Oliveras 2003) and pollination (Visser et al. 1996, Blancafort & Gómez 2005), which in turn produce significant disruptions in ecosystem assemblages.

Attempts to eradicate the invasion from affected natural areas without eliminating the queens, which constitute the reproductive power of the colony, have met with little success (Krushelnycky & Reimer 1998a, 1998b). Moreover, these methods have employed chemical procedures that are banned in some protected natural areas, making the invasion still more difficult to deal with.

As the probability of success in eradicating an invasive species decreases with an increase in its distribution range (Myers et al. 2000), the eradication of the Argentine ant in natural habitats over hundreds of hectares is practically impossible. In such places, slowing its rate of spread by making the elimination of queens the main objective of any control method applied may be the best way to manage the invasion. This is no easy matter due to the species' highly polygynous nature (the presence of more than one queen in each nest) (Keller 1995) and its unicoloniality (Passera 1994), where a lack of territorial boundaries allows it to create a large network of interconnected nests. Successfully eliminating Argentine ant queens in natural invaded environments in a more efficient way requires detailed information about their invasive process related to its physiological and ecological traits. In that sense, we wonder if colonies in the invaded-front of the introduced range may use more efficient colonising strategies to increase their invasive potential than those in the invaded zone and additionally if these strategies have changed in reference to colonies in their native range. If that is the case, the application of control methods to eliminate queens in the invaded-front may be more effective than their application in the invaded zone. The differences between the two ranges studied could help us to better understand if these are an adaptation to improve invasiveness processes in natural areas.

Until now there have been few studies of the dynamics of Argentine ant queens in natural environments that could help us answer these questions. Ingram (2002b) observed that nests in the invaded-centre of an invasion contained more queens than those in the invaded-front. Keller et al. (1989a) detected an annual execution of queens carried out by their own workers in May, and Abril et al. (2008b) observed that, due to this and nest aggregation in winter and nest splitting in summer, Argentine ant queen densities in nests vary seasonally, being higher in winter through the creation of the so-called "winter colonies", and lower in spring and summer, through the creation of the "summer colonies", characterized by their small size and lower density of workers and queens (Newell & Barber 1913, Markin 1970, Benois 1973, Heller & Gordon 2006, Abril et al. 2008b).

Unfortunately, there is still a lack of information regarding Argentine ant invasion strategies in relation to the queens. This study is an attempt to compare different traits of the biology and physiology of the queens in nests situated in the native range and in the introduced range, at the forefront of expansion (invaded-front, in contact with the native ant population) and in the totally invaded zone (invaded-centre, where the Argentine ant is almost the only ant present). This could help us to better understand the invasion process in natural environments and know whether the energy investment of the colony has changed in the process to becoming an invasive species. Specifically, we want to (1) examine for differences in Argentine ant queen densities in nests from the native range and the two areas (invaded-front and invaded-centre) of the introduced range, (2) examine for differences in the fat content of queens, (3) examine for differences in the queen/worker thorax volume ratio and (4) compare queen oviposition rates between invasion sites. All these parameters are good estimators of the invasive capabilities of the colonies, as they are directly related to dispersion speed and successfully colony foundation (Hölldobler & Wilson 1977, Keller & Passera 1989, Stille 1996, Bruna et al. 2011). According to several studies, the native range and the invaded-front may offer more available nest sites and food resources (Suarez et al. 1998, Ingram 2002b, Tillberg et al. 2007). In contrast, there is a greater competition pressure for these available resources as a result of the native ant community resisting the invasion, and the additional pressure from their own intraespecific competition (Heller 2004), natural predators and parasites in their native range (Orr et al. 2001, Tsutsui et al. 2001, Holway et al. 2002b, Reuter et al. 2005, Vogel et al. 2009). In addition, it seems that increasing queen numbers in a nest is a colony’s way to fill empty niches quickly when the environment has a strong competitive pressure (Hölldobler & Wilson 1977). On the other hand, the fat content of queens and the queen/worker thorax volume ratio seem to be related with a successful colony foundation (Keller & Passera 1989, Stille 1996). A higher fat content of queens could be an Argentine ant skill to increase their survival and, hence, achieve a successful colony foundation in highly competitive areas. Higher indexes of queen/worker thorax volume ratio seem to indicate queens with bigger thoraxes (Stille 1996), and thus, higher long-range dispersals (Bruna et al. 2011). Against this background, we hypothesised that the number and fat content of queens, and queen/worker thorax volume ratio should be following a gradual gradient through the three studied areas, from higher to lower values as follows: native range, invaded-front, and invaded-centre. The oviposition rate is closely linked to the number of queens per nest, therefore we expected a higher oviposition rate in queens from the invaded-front than in queens from the invaded-centre.

2. MATERIAL AND METHODS

2.1 Study areas

The dynamic in Argentine ant colonies vary seasonally (Newell & Barber 1913, Markin 1970, Benois 1973, Heller & Gordon 2006, Abril et al. 2008b) in both native and introduced ranges (Vogel et al. 2009). In winter, nests contain a greater density of queens and individuals as a result of a regrouping process, and in spring they split into smaller nests, with a lower density of queens and workers, as a result of a dispersion process. These are the winter nests that contain the clues to the species' dispersion power in spring and the invasion of new habitats. For this reason all the samples obtained in the present study were taken from winter nests.

2.1.1 Introduced range

The samples were taken from three different Argentine ant invasion limits (referred to as fronts of invasion) in invaded cork oak secondary forests: two located at the southern edge of the Gavarres massif, in the areas of Santa Cristina d'Aro (CA, 41º 48' 51.71''N; 3º 01' 50.57''E), and Pedralta (PD, 41º 47' 31.53'' N; 2º 58' 52.79'' E), and one in the Cadiretes massif, in the area of Puntabrava (PB, 41º46'13.51"N, 3º00'17.93"E) in the northeast (NE) of the Iberian Peninsula **(Figure 1)**. The three zones were at least 7 km apart from each other. This region has a Mediterranean climate with 690 mm of mean annual rainfall and a mean temperature of 15.5ºC.

We defined the invaded-front as where both Argentine ants and native ants are in contact, and the invaded-centre as where we found a high abundance of Argentine ants and a low presence of native ants. The contact and the invaded zones of all three localities were in close proximity (≈1.5 km apart) and had similar environmental characteristics, which meant that possible differences in terms of nest density would not be related to abiotic differences between the two zones.

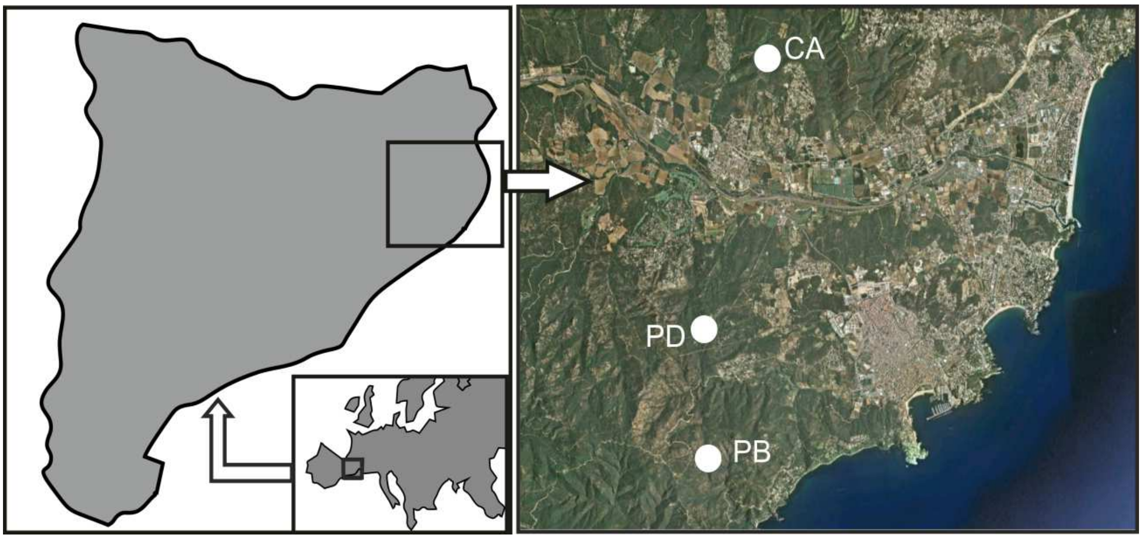


Figure 1. Location of the three study areas of the invaded range (CA: Santa Cristina d'Aro front, PD: Pedralta front and PB: Puntabrava front), Spain. (source: Google^{TM} earth 2012)

2.1.2 Native range

The study in the native range was carried out in the austral winter of 2011 (July and August) in the Natural Reserve of Otamendi (RNO, 34°14′03″S 58°53′10″O), approximately 50 km north of Buenos Aires, Argentina. It was conducted in Pampean wetlands along the first 2 km of the Islas Malvinas road that joins the railway station Ingeniero R. Otamendi to the Paraná River (Wild 2004, Pedersen et al. 2006, Vogel et al. 2009). Winter nests were searched in both sides of the road, at approximately 4-m-wide strip of vegetation between the road and the canals running parallel to it **(Figure 2).** This region has a Mediterranean climate with a mean annual rainfall of 1021 mm and a mean temperature of 16.3ºC.

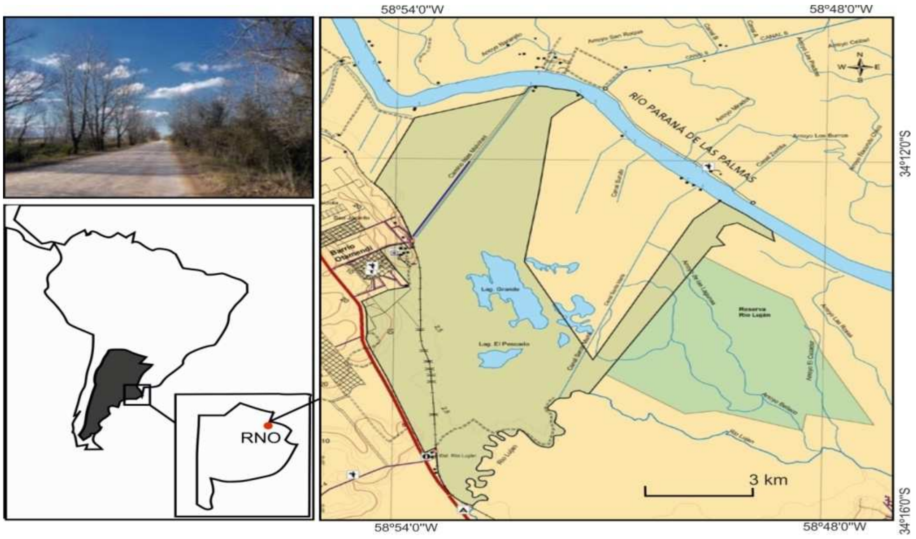


Figure 2. Native studied area in the north of Buenos Aires, Argentina. Figure on the bottom left represents the geographical province of Buenos Aires; the red circle indicates the location of the Natural Reserve of Otamendi (RNO in Spanish). Figure on the right represents the RNO (source: http://www.ambiente.gov.ar/?idarticulo=5345); dark blue line indicates the 2 km section of the area sampled on the Islas Malvinas road and picture (F. Fatú) on the top left represents the native area sampled of these 2 km section.

2.2 Supercolonies in the native range

In order to characterize the Argentine ant population in the Natural Reserve of Otamendi, we followed the same procedure as Vogel et al. (2009). We conducted aggression tests between pair of workers from adjacent nests to determine the number of distinct supercolonies present along the first 2 km of the road sampled from the railway station to the Paraná River (**CHAPTER 1**). Like Vogel et al. (2009), we considered a “supercolony” to be each group of nests linked with a complete lack of aggression between each other, but with a very high aggression among supercolonies. Number, distribution and density of supercolonies in the native range were assessed as in **CHAPTER 1, section 2.2**. We evaluated queen density, queen/worker thorax volume ratio and fat content in queens between each supercolony found.

2.3 Queen density in native and introduced ranges

We estimated Argentine ant queen density per litre of nest soil, taking samples of two litres of soil from 24 nests in each locality of the native (Otamendi) and the introduced ranges (Santa Cristina d’Aro, Pedralta and Puntabrava). In the invaded localities 12 of these nests were from the invaded-centre, and the other 12 were from the invaded-front.

In total, we sampled 72 litres of soil from 36 different nests in each zone of the introduced range (invaded-front and invaded-centre) and 48 litres of soil from 24 different nests in the native range. Nests in the introduce range were taken in the boreal winter of 2010 (January to March) and nests in the native range were taken in the austral winter of 2011 (July to August). Once in the laboratory, we manually extracted the queens present in each sample.

2.4 Queen/worker thorax volume ratio in native and introduced ranges

We collected a total of 300 queens and 300 workers from eight different nests in the invaded-front and 290 queens and 290 workers from 28 different nests in the invaded-centre of the introduced range, and 90 queens and workers from 17 different nests in the native range. We made a dried collection of these specimens and then we estimated the worker and queen thorax volumes (length x width x height) in order to determine the queen/worker thorax volume ratio, as in Stille (1996).

2.5 Fat content of queens in native and introduced ranges

We collected queens from nests situated in the native range and in the invaded-front and the invaded-centre. We collected a total of 44 queens from 10 different nests in the native range and 161 queens from 15 different nests and 131 queens from 10 nests in the invaded-front and invaded-centre, respectively. We killed them with ethyl acetate vapor in the laboratory and then followed the same procedure as Keller & Passera (1989) based on Peakin (1972). We first determined the individual fresh weight of queens, and then dried them at 70ºC for 24 hours. After that, we determined their dry weight and extracted the fat with petroleum ether (boiling point 40-60ºC) and a Soxhlet apparatus over 24 hours. The queens were then dried again at 70ºC for 24 hours and weighed. All weights were determined to the nearest 10^{-5} g. Fat content was expressed as a percentage of dry weight.

2.6 Daily oviposition rate of queens in the introduced range

In order to asses if there were differences between the oviposition rates of queens in nests in the contact and the invaded zones, we collected in both Santa Cristina d’Aro and Pedralta a total of 24 queens from six different nests in each zone (four queens per nest). In the laboratory, we created six artificial polygynous nests with four queens for both the contact and invaded zones. Each nest had the same ratio of workers to queens—approximately 200 workers per queen (800 workers per nest). All the queens/workers in the artificial nests came from the same nest in the field. The artificial nests were made up of a regular plastic box (180 mm x 115 mm and 35 mm high) fitted with a layer of dry plaster of Paris connected laterally to a smaller box (75 mm x 50 mm and 25 mm high) by a cotton-wool wick permanently in contact with a piece of cotton soaked in water. To prevent escape, the inner sides were coated with liquid PTFE (Fluon ®). The ants were fed daily with a variant of the artificial diet described by Keller et al. (1989b), reported in Abril et al. (2008a), which allowed a healthy production of workers and sexuals and high fecundity in queens (Abril et al. 2008b, 2010). The nests were incubated at 28ºC, the optimal temperature for queen oviposition in the Argentine ant (Abril et al. 2008a). After one week of incubation in the laboratory, the queens were subjected to an oviposition test that followed the same procedure as in Abril et al. (2008a). In brief, this consisted of isolating the queens individually and some of their workers (3-5 workers) in test-tube nests for 24 hours. After this period we counted the eggs laid by each queen. The test-tube nests were plastic tubes that used the same mechanics as the nests described above to provide humidity to the individuals. They were 70 mm long x 10 mm in diameter, with a plastic top covered on its inner side by a layer of dry plaster of Paris connected by a wick of cotton wool to a small chamber filled with water. In order to be able to identify each queen throughout study, we marked them with Uni Paint marker pens (Mitsubishi Pencil Co., LTD.) on the dorsal surface of their thorax. The oviposition rate of each queen was measured every four days, for a total of six times. The duration of the study was one month, from March to April 2009, comprising the period when the oviposition of the queens was at its maximum (Benois 1973).

2.7 Data analysis

We made all the comparisons between queens from the native range and the contact and invaded zones of the introduced range and between supercolonies, using generalised linear mixed models (GLMMs).

Comparisons between queen densities per litre of soil, fat content of queens and oviposition rate was assessed using GLMMs with a Poisson error distribution and a log link function. In the case of queen densities per litre of soil, the area (Santa Cristina, Pedralta, Puntabrava or Otamendi) was used as a random factor and the zone (native, invaded-front or invaded-centre) as a fixed factor. In the case of fat content of queens and the oviposition rate we used as a random factor the nest and the zone (native, invaded-front or invaded-centre) as a fixed factor. Comparisons between queen/worker thorax volume ratio and supercolonies in the native range were performed using GLMMs with a Gaussian error distribution and an identity link function. In the case of fat content and queen/worker thorax volume ratio, the nest was used as a random factor and the zone (native, invaded-front or invaded-centre) as a fixed factor. In the case of supercolonies we used as a random factor the nest and the supercolony as a fixed factor.

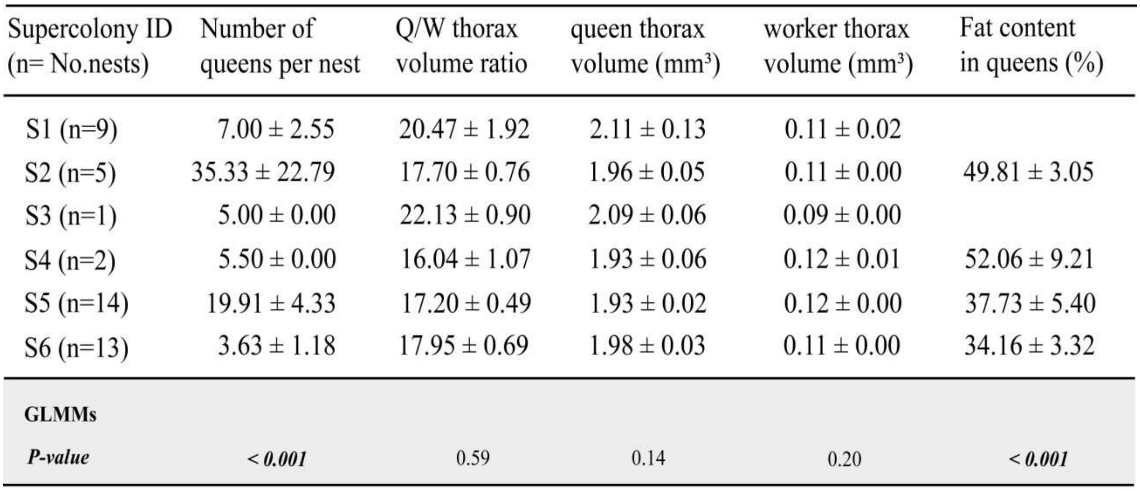
We also used lineal regressions to evaluate the relationship between the characteristics of each supercolony and queen density, Q/W thorax volume ratio, thorax volume of queens and workers, and fat content of nests in the native range. All statistics were calculated using the R 2.12.1 for Windows package (R Development Core Team 2010) for all analyses. Queen density was log-transformed to achieve normality in lineal regressions.

3. RESULTS

3.1 Supercolonies in the native range

We found six supercolonies, whose location would match with those found by Vogel et al. (2009), along the first 2 km sampled on the two sides of the road from the railway station to the Paraná River. The characteristics of each of the six supercolonies are given in **Figure 3** and **Table 1** of **CHAPTER 1**. Energy investment in queens of each supercolony present in the native range was summarised in **Table 1**.

Table 1. Energy investment in queens of the Argentine ant supercolonies at the Natural Reserve of Otamendi (n=No. nests, refers to the number of distinct nests of each supercolony) (mean ± SE). GLMMs (Generalised Linear Mixed Models) showing differences between native supercolonies. *P-value* in bold italic is a significant value (*P < 0.05*).



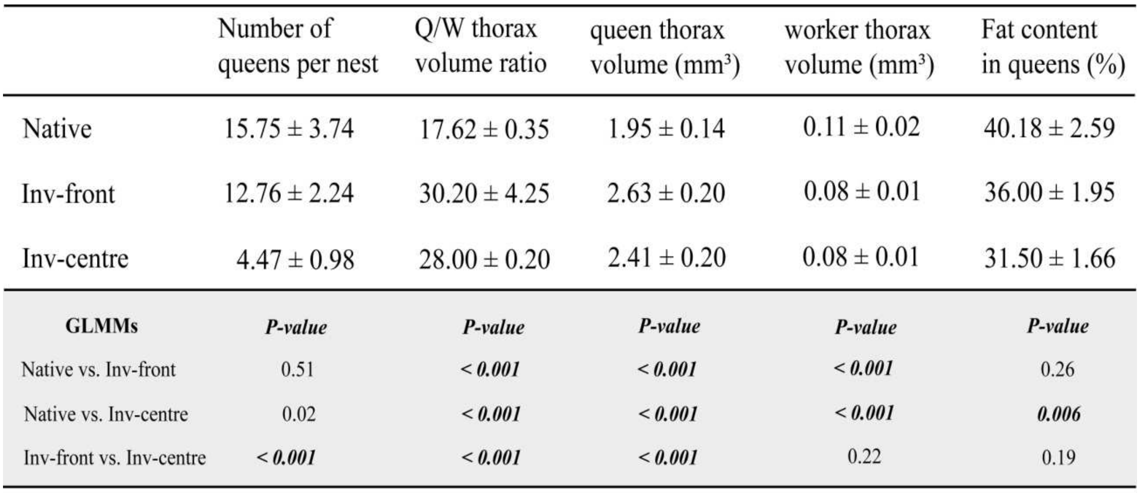
Supercolony ID (n= No.nests)	Number of queens per nest	Q/W thorax volume ratio	queen thorax volume (mm^3)	worker thorax volume (mm^3)	Fat content in queens (%)
S1 (n=9)	7.00 ± 2.55	20.47 ± 1.92	2.11 ± 0.13	0.11 ± 0.02	
S2 (n=5)	35.33 ± 22.79	17.70 ± 0.76	1.96 ± 0.05	0.11 ± 0.00	49.81 ± 3.05
S3 (n=1)	5.00 ± 0.00	22.13 ± 0.90	2.09 ± 0.06	0.09 ± 0.00	
S4 (n=2)	5.50 ± 0.00	16.04 ± 1.07	1.93 ± 0.06	0.12 ± 0.01	52.06 ± 9.21
S5 (n=14)	19.91 ± 4.33	17.20 ± 0.49	1.93 ± 0.02	0.12 ± 0.00	37.73 ± 5.40
S6 (n=13)	3.63 ± 1.18	17.95 ± 0.69	1.98 ± 0.03	0.11 ± 0.00	34.16 ± 3.32
GLMMs					
P-value	***< 0.001***	0.59	0.14	0.20	***< 0.001***

The mean queen density per litre of nest soil found in each supercolony of the native range was very variable: from 1.00 to 79.50 queens/l, and differed within each other, as well as fat content in queens. Moreover, supercolonies containing more nests, and therefore larger ones, had a higher queen density per litre of nest soil (r^2 = 0.187, P = 0.035) and invested less in queen fat reserves (r^2 = 0.15, P = 0.008). The mean queen/worker thorax (Q/W) volume ratio and mean queen and worker thorax volumes in the native range did not differ among supercolonies.

3.2 Queen density in native and introduced ranges

Queen density per litre of nest soil for each of the three sampling areas (native, invaded-front and invaded-centre) are summarised in **Table 2**.

Table 2. Values for energy investment in queens in terms of queen density, Q/W thorax volume ratio, queen and worker thorax volume (mm^3), and fat content in queens (%) (mean ± SE). GLMMs (Generalised Linear Mixed Models) showing the differences between each sampling zone. *P-value* in bold italic is a significant value ($P < 0.05$). Inv-front: invaded-front, Inv-centre: invaded-centre.



	Number of queens per nest	Q/W thorax volume ratio	queen thorax volume (mm³)	worker thorax volume (mm³)	Fat content in queens (%)
Native	15.75 ± 3.74	17.62 ± 0.35	1.95 ± 0.14	0.11 ± 0.02	40.18 ± 2.59
Inv-front	12.76 ± 2.24	30.20 ± 4.25	2.63 ± 0.20	0.08 ± 0.01	36.00 ± 1.95
Inv-centre	4.47 ± 0.98	28.00 ± 0.20	2.41 ± 0.20	0.08 ± 0.01	31.50 ± 1.66
GLMMs	*P-value*	*P-value*	*P-value*	*P-value*	*P-value*
Native vs. Inv-front	0.51	***< 0.001***	***< 0.001***	***< 0.001***	0.26
Native vs. Inv-centre	0.02	***< 0.001***	***< 0.001***	***< 0.001***	***0.006***
Inv-front vs. Inv-centre	***< 0.001***	***< 0.001***	***< 0.001***	0.22	0.19

Queen density per litre of nest soil in the native range did not differ from that of the invaded-front, but was much higher than that of the invaded-centre. Moreover, queen densities per litre of nest soil were higher in the invaded-front than in the invaded-centre (**Figure 3**). Additionally, the number of workers per litre of soil in nests with more queens as in the native range and the invaded-front also seemed considerably higher than the ones in the invaded-centre with low number of queens per nest (personal observation).

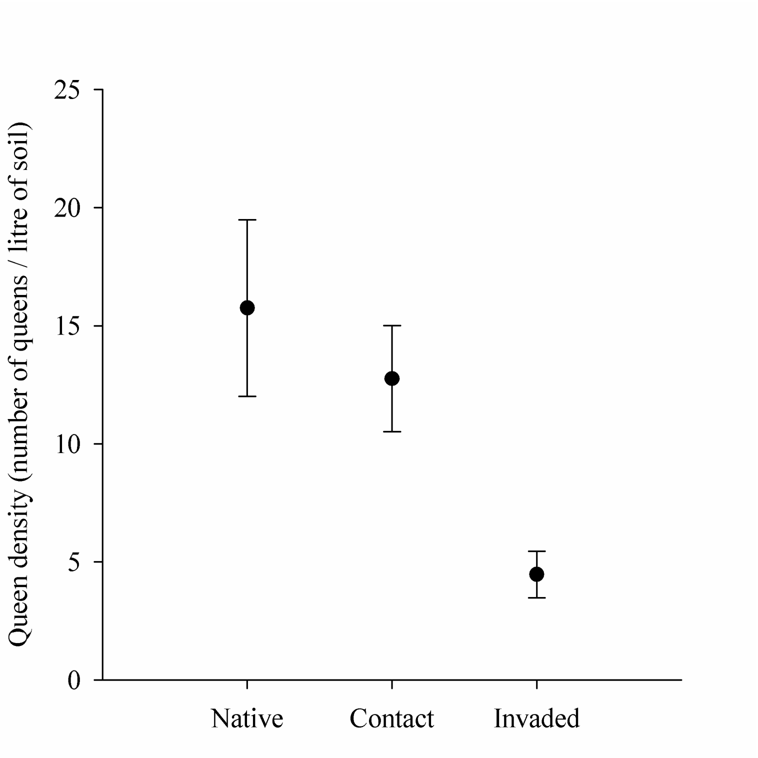


Figure 3. Queen density (mean ± SE) measured as number of queens per litre of soil in the native range (n=24) and the two zones (invade-front and invaded-centre, both n=36) of the introduced range. Contact: invaded-front, Invaded: invaded-centre.

3.3 Queen/worker thorax volume ratio in native and introduced ranges

There were significant differences between the queen/worker (Q/W) thorax volume ratios in the native range and the two zones of the introduced one **(Table 2)**.

The mean Q/W thorax volume ratio in the native range was lower than in the invaded-front and in the invaded-centre. Moreover, the Q/W thorax volume ratio was higher in the invaded-front than in the invaded-centre **(Figure 4)**.

These results were due to differences in thorax volumes in queens **(Table 2).** Queens in the native range were smaller than those in the invaded-front and the invaded-centre of the introduced range

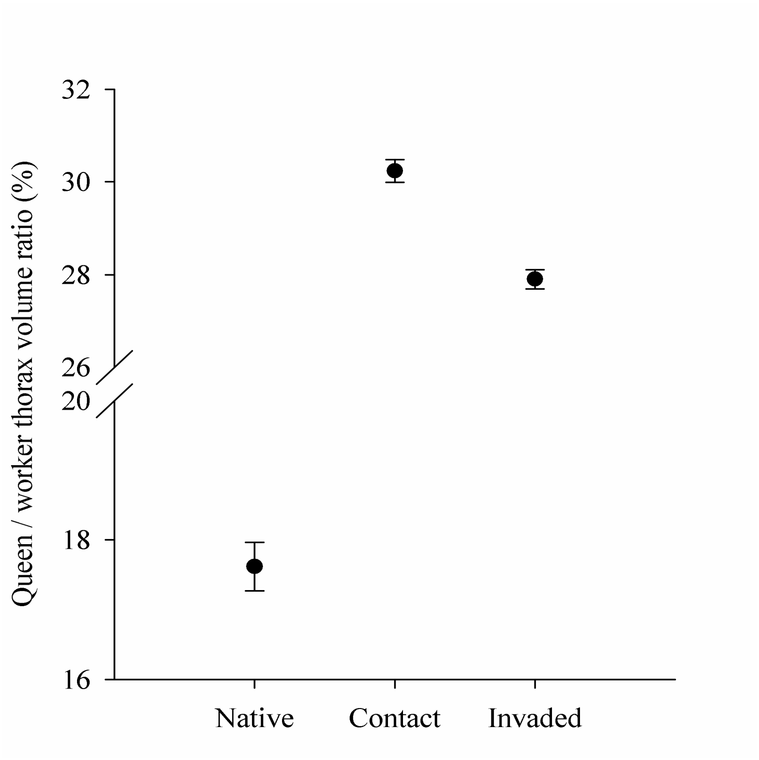


Figure 4. Q/W volume ratio (mean ± SE) in the native range (n=89) and in the two zones (invaded-front, n=300 and invaded-centre, n= 290) of the introduced range. Contact: invaded-front, Invaded: invaded-centre.

Additionally, queens in the invaded-front were bigger than those in the invaded-centre **(Figure 5a).** However, the thorax volumes of workers were the same for the two zones of the introduced range, while they both were smaller than in the native range **(Figure 5b).**

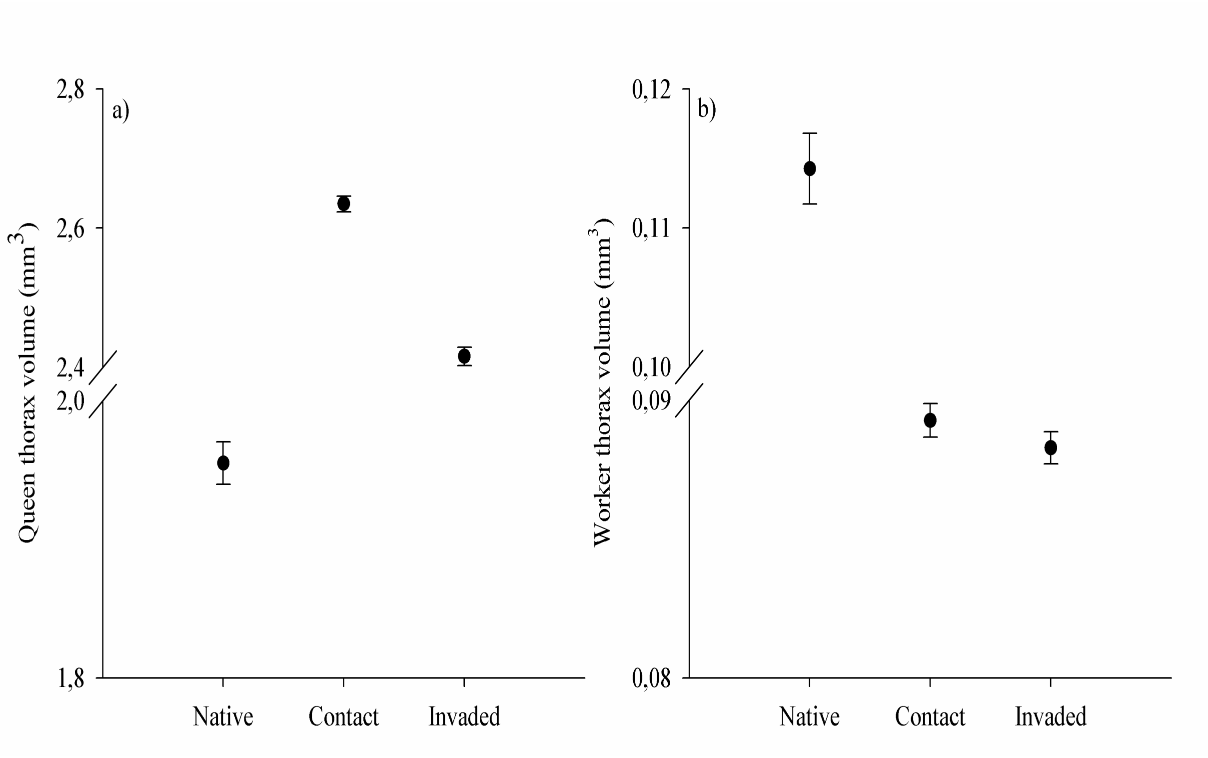


Figure 5. Volume measurements (mean ± SE) of (a) Argentine queens and (b) Argentine workers in the native range (n=89) and the two zones (invaded-front, n=300 and invaded-centre, n=290) of the introduced range. Contact: invaded-front, Invaded: invaded-centre.

3.4 Fat content of queens in native and introduced ranges

The mean fat content in queens' results shows a gradient of fat reserves investment from the native to the most invaded zone **(Table 2)**. The percentage of fat content in queens in the native range was the same as in the invaded-front, but it was much higher than in the invaded-centre. However, colonies of the invaded-front and the invaded-centre of the introduced range invested the same in queen fat reserves **(Figure 6)**.

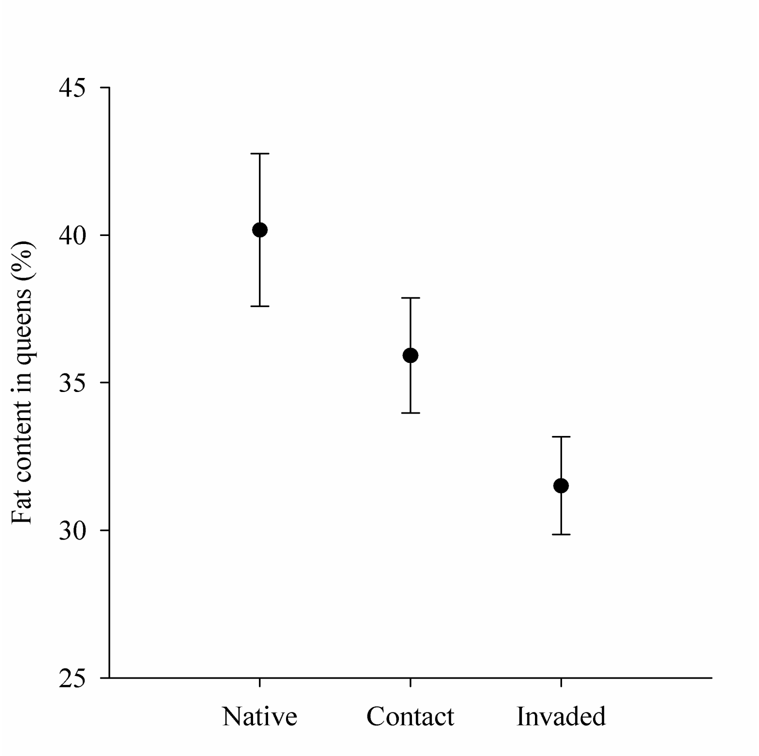


Figure 6. Fat content in queens (mean ± SE) of the native range (n=44) and the two zones (invaded-front, n=131 and invaded-centre, n=161) of the introduced range. Contact: invaded-front, Invaded: invaded-centre.

3.5 Daily oviposition rate of queens in the introduced range

The daily oviposition rates of queens from both zones of the introduced range were not significantly different (GLMM: P (|>χ^2|) = 0.57) **(Figure 7).**

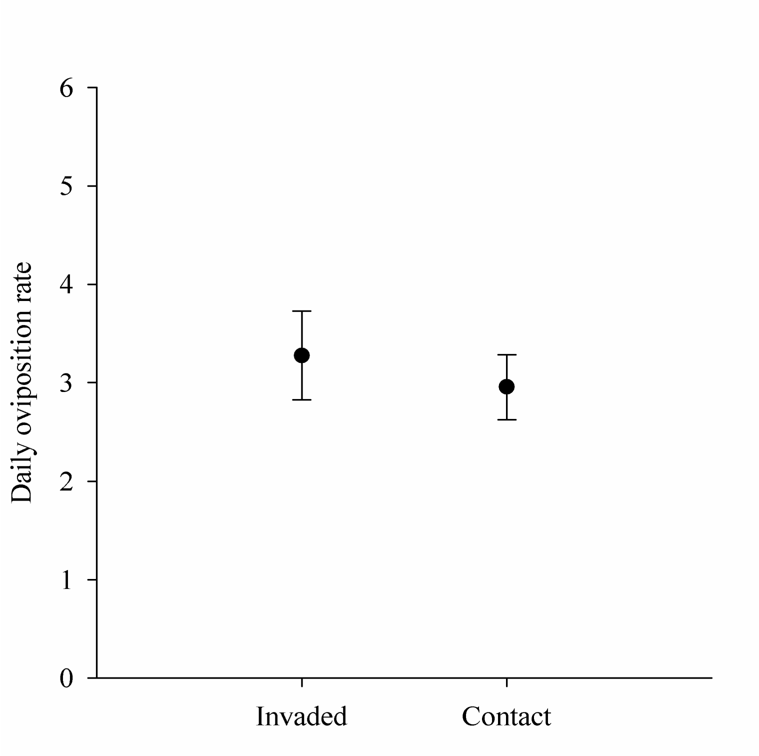


Figure 7. Daily oviposition rate of queens (mean ± SE) in both zones (invaded-front, n=144 and invaded-centre, n=137) of the introduced range. Contact: invaded-front, Invaded: invaded-centre.

We also calculated the individual contribution of each queen to the daily oviposition of the whole artificial nest, and observed that in all cases it was always the same queen that contributed most **(Figure 8).**

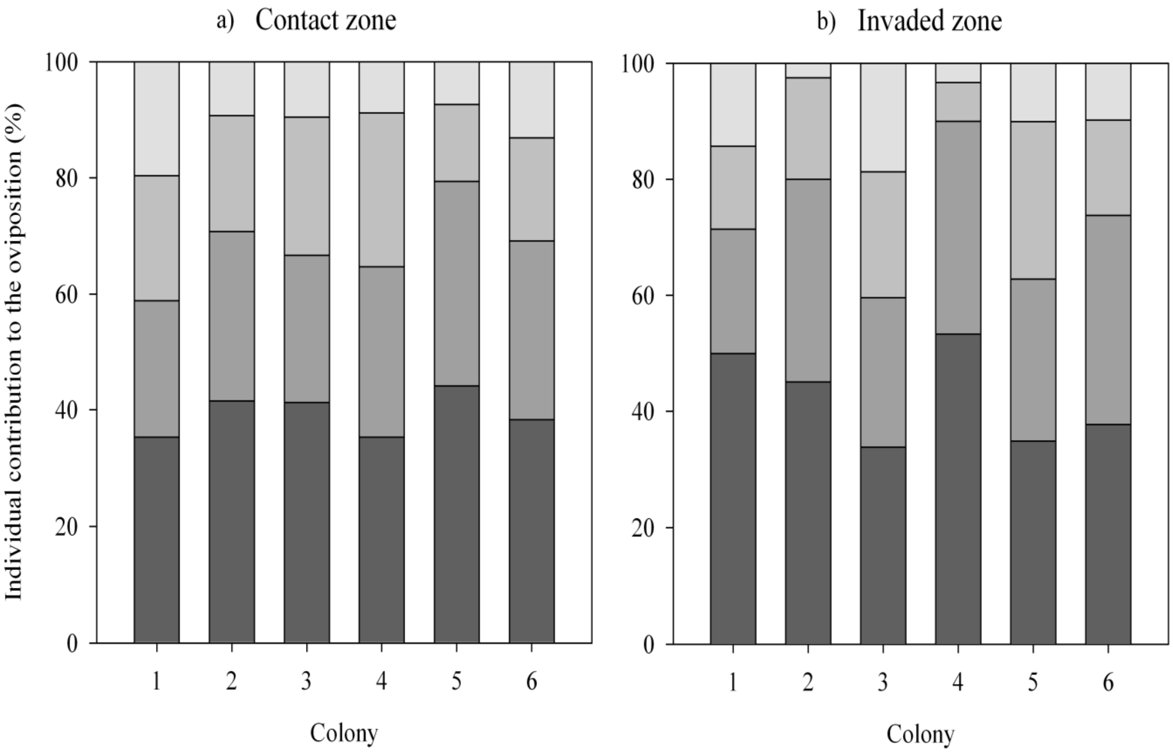


Figure 8. Individual contribution to the oviposition of the colony (%) from (a) nests situated in the contact zone (invaded-front) of the invasion and (b) nests situated in the invaded zone (invaded-centre) of the invasion. Each colour of each stack represents the proportion of eggs laid by one of the four queens of the artificial nest (colony) in relation to the total of eggs laid by the four queens in 24 hours.

4. DISCUSSION

According to Keller & Passera (1988), colony investment in gynes can be altered by changing the energy content of each gyne and/or by changing the number of gynes produced. The findings of this study suggest that Argentine ant colonies situated in the native range and the invaded-front of a natural area make the same investment of energy in gynes in terms of number of queens. These colonies have more queens than colonies situated in the invaded-centre. It seems that high queen numbers can be considered as a colony’s way of adapting to the need to fill empty niches quickly (Hölldobler & Wilson 1977). Thus, these differences in queen densities may be an adaptation to the ecological differences between these zones. The native and the invaded-front may offer more available nest sites and food resources than the saturated habitat of the invaded-centre (Ingram 2002b). In addition, the well-structured community of ants in the native and the invaded-front force the Argentine ant to compete for food resources and nesting sites with other native ant species. In their native range approximately 51 ant species (Cabrera 2009) compete against the Argentine ant, some of which are highly dominant. The same occurs in colonies of the invaded-front, where they have to compete with the native ant species resisting the invasion (Casellas 2004), consisting of approximately 17 species (M.L. Enríquez, S. Abril, M. Diaz & C. Gómez, unpubl.), some of which are also highly dominant (Cerdà et al. 1997). On the contrary, in the invaded-centre there is less pressure on colonies to compete for food and nesting sites. This is because the only ant species remaining in the invaded-centre that is abundant enough to compete with the Argentine ant is *Plagiolepis pygmaea* (Latrielle 1869) (Oliveras et al. 2005), a subordinate species (Cerdà et al. 1997) which coexists with the invader in apparent equilibrium (Abril & Gómez 2009). Under these conditions, colonies in their native range and the invaded-front may increment their queen densities in order to increase their chances of success in colonising new areas. However, we expected to find a higher number of queens in the native range than in the invaded-front, as the Argentine ant in the former is additionally submitted to the pressure of intraespecific competition, natural predators and parasites (Orr et al. 2001, Tsutsui et al. 2001, Holway et al. 2002b, Reuter et al. 2005, Vogel et al. 2009). So, a higher number of queens per nest could increase their survival and achieve a successful colony foundation in this highly competitive area. Nevertheless, another possible explanation for the increased number of queens in the native and the invaded-front could be that interspecific competition for nesting sites left less suitable places to nest in winter, forcing the Argentine ant to fuse more summer nests, thus, a greater number of queens and workers, in a small number of winter nests.

Our results in regard to queen densities in the introduced range do not agree with those obtained by Ingram (2002b) in an invaded natural area of the Haleakala National Park in Hawaii. This may be due to the sampling period. While we took samples in winter to coincide with the period of higher queen densities in the nests (Abril et al. 2008b), Ingram (2002b) collected the nests later, likely sampling summer colonies, which are smaller and have a lower density of queens (Abril et al. 2008b). This could explain why she found mainly monogynous nests in the contact zone of the invasion, probably because they had recently been founded by queens who had abandoned their mother nests. Differences between the two studies could also be a consequence of different ecological contexts in the respective contact zones of the invasion. Unicolonial species such as the Argentine ant form colonies of many small nests with few queens in areas where there is little competition or predation, whereas in more competitive environments, larger nests are built because they offer more protection from colony mortality (Hee et al. 2000). The Haleakala National Park is characterised by its relatively few competitive constraints, since the native ant population coexisting with the invader is formed by only two species, and neither is particularly aggressive (Ingram 2002b). This could explain why in this area nests in the contact zone of the invasion had fewer queens and were mainly monogynous.

On the other hand, it seems that in social insects the size of reproductive individuals is associated with dispersal capabilities. Thus, larger-sized queens are associated with long-range dispersal and vice versa (Bruna et al. 2011). Taking this into account, colonies in Otamendi and in the invaded-front of the introduced range, which have a high competition pressure from other native ants, are supposed to invest more energy in creating larger queens so as to ensure new colonies will be established more quickly in these areas following dispersal from the mother nests in spring. Although this assumption could fit with colonies in the invaded-front, queens in the native range were smaller than those in both zones of the introduced one. This may be due to that the common intraspecific aggression in the native range makes them reduce their interspecific competitive ability. As a result of this, the Argentine ant should coexist with numerous species of ants in their native range and not be as numerically dominant as they are in the introduced one (Holway et al. 2002a). Moreover, they may even not need such a long-range dispersal as the invasive colony of the introduced range. But this is only an assumption, and further research on the relation between bigger queens and longer dispersal on foot among Argentine ant queens is needed to contrast this idea. Another possible and non-excluding explanation of these differences in the abundance and size of gynes could be related to the reduction of N-rich protein sources in the areas situated behind the front of invasion and in their native range, affecting the production of queens through the reduction of their protein intake (Tillberg et al. 2007).

On the other hand, as suggested by Keller & Passera (1988) in the case of dependent mode of colony founding, reproduction investment per colony not only depends on reproductive units, but also on the amount of workers which leave the nest with the queen(s). They also suggest that larger species often produce lower numbers of workers than smaller ones during colony founding (Keller & Passera 1989). We found that in its native range the Argentine ant produces smaller queens and larger workers than in both zones of the introduced range. One explanation for this could be that colonies from invaded areas produce smaller workers, as they are less costly energetically. So the species could reproduce more and faster, creating a higher number of workers in these areas (Holway et al. 2002a), which give them a higher invasiveness potential (Heller et al. 2008b). Another possibly explanation could be that colonies in the native range invests more resources to rear the workers that will later follow the queen when she leaves the mother nest than in gynes. But more studies about morphology in workers are needed to answer whether differences in worker size between both the native and the introduced range could be a strategy to increase success in invasiveness processes in introduced areas or/and a strategy to increase success when they found new colonies in the native range.

Data on the fat content of mature Argentine ant gynes reveal that although values in the introduced range are relatively high for a dependent colony founding queen, they fall within the range of data for ants of this kind, while queens in the native range go above the dependent colony founding threshold suggested by Keller & Passera (1989). This high variability in fat content of mature queens in the Argentine ant could be due to different second adaptations to nest establishing strategies in each range. Keller & Passera (1989) proposed that large queens may need lower amount of energy in proportion to their weight for their own metabolism. Additionally, larger queens often produce lower number of workers than smaller ones during colony founding, and therefore need less fat reserves to rear them (Keller & Passera 1989). Considering this, larger and less fatty queens in the invaded zone should produce a lower number of workers than those smaller and fattier queens from the native area. Instead of it, workers in the introduced range are smaller and maybe more numerous (personal observation) than those workers in the native range. Given this, the Argentine ant could be producing larger queens in the introduced range which, according to Keller & Passera (1989), need less fat reserves for their own, and thus, part of these queen fat reserves could be invested in producing smaller workers but a great number of those. Therefore, it seems that the Argentine ant may be doing potential shifts in some of its biological strategies (i.e. size of queens and workers, and fat content in queens) linked to the increasing of its invasiveness in the introduced range. Additionally, the Argentine ants' thorax volume ratio in both zones of the introduced range is quite high, indicating that although they have a dependent mode of colony founding (Hee et al. 2000), their queens' morphology is best fitted to one of independent colony founding (Stille 1996). This suggests that queens in both the invaded-front and the invaded-centre of the introduced range are morphologically well adapted to carrying out independent colony founding (as their queen/worker thorax volume ratios indicate), but physiologically they are not, indicating there is a halfway point between independent and dependent colony founding. Curiously, similar results were found for the invasive ant *Lasius neglectus* (Van Loon, Boomsma & Andrasfalvy 1990). This species' colony founding mode is dependent, but it is morphologically qualified to carry out independent colony founding (Espadaler & Rey 2001).

However, the Argentine ant in its native range could be physiologically and morphologically well adapted to carrying out independent colony founding (as queen/worker thorax volume ratios and percentage of fat content indicate). As there are no studies reporting the mode of colony founding of native colonies of the Argentine ant, could these differences between ranges be a result of shifts in the colony's strategies to become an invasive species in the introduced range? And moreover, could the coincidence in results with *L. neglectus* be the result of the invasion processes of these species in each of their introduced ranges? To shed light on these questions, further research on the mode of colony founding of these two species (*L. humile* and *L. neglectus*) in their natural ranges is needed.

There are no differences between the oviposition rates of queens from nests in the two zones of the introduced range. It seems that an increase in their reproduction capabilities is not a decisive factor in the quicker colonisation of new habitats. Moreover, there is always a single queen that contributes most to the oviposition of the colony irrespective of the zone. A previous study on the fecundity of Argentine ant queens proved that in polygynous nests there was always a queen that contributed proportionately more to egg laying than the others (Abril et al. 2008a), but the study did not answer the question of whether or not this queen was always the same queen. We have resolved that question in this study, but further research is necessary to understand the mechanisms that make that particular queen more fertile than the others.

To sum up, the ecological and morphological differences found in the present study regarding the Argentine ant queens seems to respond to the different ecological contexts between the three zones studied (native, invaded-front, and invaded-center). The different strategies adopted in each zone would provide the colonies with different skills to compete for available resources and to assure a successful new colony founding. These skills are, a higher intra- and interspecific competitive ability in the native range, and a higher invasiveness potential in the invaded-front of the introduced range. Those skills give to colonies the capability to compete for empty niches and available resources in each of these zones.

CHAPTER 3. Assessment of the Argentine ant invasion management by means of manual removal of winter nests in mixed cork oak and pine forests

(picture: M. Diaz)

Assessment of the Argentine ant invasion management by means of manual removal of winter nests in mixed cork oak and pine forests

1. INTRODUCTION

Native to South America, the Argentine ant (*Linepithema humile*, Mayr 1868) has been introduced worldwide into areas with Mediterranean-type climates due to human commercial activities (Hölldobler & Wilson 1990, Passera 1994, Suarez et al. 1998, Suarez et al. 2001, Roura-Pascual et al. 2011). One of the keys to the success of this invasive ant is the social behaviour of its colonies based on unicoloniality (Passera 1994). Unicolonial ant species are characterized by a large number of nests typically containing many queens and workers coexisting, where individuals from different nests are mixed and intraspecific aggression is infrequent (Hölldobler & Wilson 1977), in contrast to multicolonial, in which each colony has a single nest and ants are aggressive against non nest-mates. Their aggressive behaviour (Carpintero & Reyes 2008) and their numerical dominance over native ants influence the outcome of competitive interactions among this unicolonial invasive species and the native species they retract (Heller et al. 2008b), and cause several negative impacts on arthropod communities (Human & Gordon 1997, Holway 1998, Suarez et al. 1998), ant-vertebrate interactions (Suarez et al. 2000, Estany et al. 2010) and ant-plant relationships (Bond & Slingsby 1984, Visser et al. 1996, Gómez & Oliveras 2003, Blancafort & Gómez 2005, Rodríguez-Cabal et. al 2009), with a drastic decrease the biodiversity of the affected areas. Because of this unicolonial structure, Argentine ant colonies are spatially, behaviourally and genetically diffuse (Heller et al. 2008a). They are characterized by the formation of "expansive supercolonies" with a regular pattern of organization and a seasonal polydomy (Heller et al. 2008a).

The nesting behaviour of Argentine ants changes according to biotic factors (e.g., surrounding plant structures, canopy cover, etc.) and abiotic factors (e.g., temperature and soil water content) and differs from summer to winter (Heller et al. 2006). The Argentine ant moves its nests in response to seasonal shifts in the environmental conditions or available food depending on the specific colony requirements in that moment (Heller et al. 2006). During the cold season of the Mediterranean-type climate areas, the spatial range of the colony is contracted and nests are combined to form the so-called “winter nests”. These nests are usually located mostly under rocks, near plant structures and facing south (**CHAPTER 1**). These characteristics help to maintain a range of temperature that permits soil moisture regulation inside the nest and allows colony activity during winter (**CHAPTER 1**). In contrast, during the hot and dry season, the spatial range of the colony is more disperse and distributed at random in smaller nests that are interconnected with long trails that cover large foraging areas. These seasonal shifts in the local distribution of nests produce a fluctuating invasion front, and consequently a fluctuating impact on native species from these zones (Heller et al. 2006, M.L. Enríquez, S. Abril, M. Diaz and C. Gómez, unpubl.).

Efforts to eradicate established populations in invaded natural areas have had little success (Silverman & Brightwell 2008). The most common control method used is toxic baits (Krushelnycky & Reimer 1998a, 1998b). This method reduces worker populations but seem to fail at killing the queens, which hold the reproductive power of the colony. In consequence, the colony has a rapid recovery of its foraging activity rates after the application of the treatment (Krushelnycky & Reimer 1998a, 1998b). Bait with sucrose and boric acid has also been assessed and showed effective control of queens in the laboratory when the bait was continuously available (Hooper-Bui & Rust 2000, Klotz et al. 2000), but there is no information on its effect in natural areas. Other studies have attempted to disrupt the foraging activity of workers with a synthetic pheromone (Suckling et al. 2008, Nishisue et al. 2010, Suckling et al. 2010), which seems to suppress worker recruitment in the short term (Tanaka et al. 2009), but the long term effect is very weak when the density of the target pest is high (Cardé 1990, Nishisue et al. 2010). Nishisue et al. (2010) concluded that the combination of toxic baits and pheromones, along with native ant competitors, could be an effective way to suppress resource acquisition by Argentine ants. However, since the use of chemicals is not allowed in some natural areas of special interest, especially wide-spectrum products that could have a negative impact over other arthropods (Suckling et al. 2008), the most sensible way to control the invasion is to slow the rate it spreads and to limit its establishment in other non-invaded areas and the consequent negative impact on the ecosystem. The manual removal of a high number of queens and their broods in the advancing invasion front could be weaken its expansion without the use of chemical procedures. The maximum queen densities in the Argentine ant nests are found in winter, from January to March, (Abril et al. 2008b). The systemic elimination of queens and workers during that period might offer a degree of resistance to the invasion, and hence, to its expansion into non-invaded areas. The aim of this study is to discover (a) the seasonal changes and the spatial distribution of the Argentine ant nests during a year, (b) the effect of manual extirpation of winter nests on the spatial dynamics of these nests year by year and on the presence of their individuals, and (c) the two-year effects of winter nests extirpation on the dispersal capacity of the Argentine ant.

2. MATERIAL AND METHODS

2.1 Study Areas

We studied populations of the Argentine ant in 18 plots throughout two consecutive winters, from December 2008 to December 2009, and two additional months in

February 2010 and December 2010, at three invaded locations of open cork oak secondary forests. Two were located on the southern edge of the Gavarres Massif: 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) and one in the Cadiretes Massif: Puntabrava (PB, 41°46'13.51"N 3º00'17.93"E) all within the NE of the Iberian Peninsula **(Figure 1)**. The three zones were at least 7 km apart from each other. This region has a Mediterranean climate with 690 mm of mean annual rainfall and 15.5ºC of mean temperature.

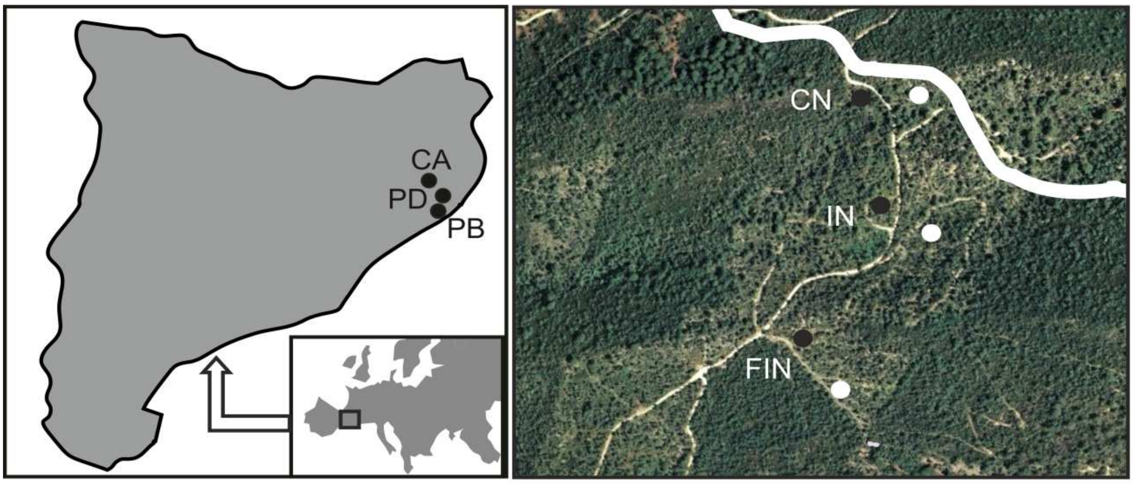


Figure 1. Invaded areas of the Argentine ant colonies in the NE of the Iberian Peninsula. Black circles indicate localities sampled (CA: Santa Cristina d'Aro, PD: Pedralta and PB: Punta Brava). The map on the right illustrates the front of the invasion (white line) and set plots of CA: Control (black dots) and extirpation (white dots) along contact (CN), invaded (IN) and fully invaded (FIN) plots. (source: Google^{TM} earth 2012)

We evaluated seasonal variation in nests during the first year of the survey (from December 2008 to December 2009), and the effect of manual extirpation in both years (from December 2008 to February 2010, and in December 2010), from the edge and along the invasion front. We carried out sampling in the field by means of mapping nests in a total of eighteen 12x12 m plots **(Figure 2)**.

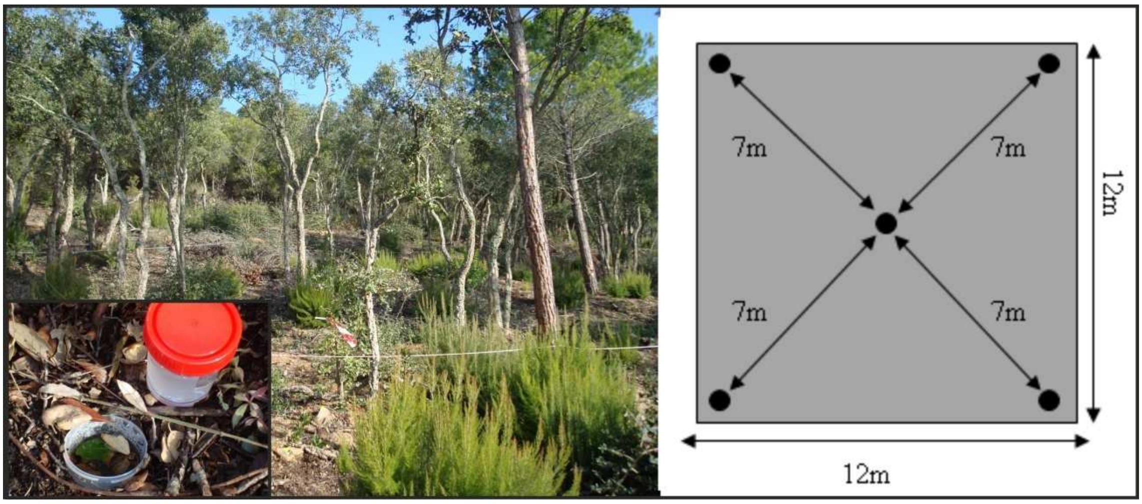


Figure 2. Picture on the left represents one sub-plot of the open cork oak secondary forest sampled and type of pitfalls used. Picture on the right represents a schematic drawing of one 12x12 m plot, illustrating the winter nests sampled area and pitfall situations (black dots). (picture: M. Diaz)

Prior to designing the study, we identified the invasion fronts of each location with bait sampling. For this purpose, tuna and marmalade baits were placed every 4 m along random transects 100 m in length, and the invasion limit was identified by the last bait visited by the Argentine ants. After identifying the front path in each locality, we defined the three areas from the front (edge) of the invasion to the most invaded area in which pairs of plots were divided into two groups according to treatment (i.e. plot type): control plots (CT) without treatment, and extirpated plots (EX) in which nests were manually removed. CT and EX plots were always separated by a human-path (i.e. walking track), at a mean distance of ≈110 m from each other. We divided each plot type in three sub-plots along the front of the invasion (i.e. plot zone): contact (CN, located at the invasion front at a mean distance of 20 m from the edge, in contact with the native ant species), invaded (IN, located in the invaded area behind the edge at a mean distance of 250 m from it, coexisting with some native ant species) and fully invaded (FIN, located well behind the edge of the invasion front at a mean distance of 450 m, coexisting with few native ant species, mostly submissive species such as

Plagiolepis pygmaea (Latreille 1798) or *Camponotus lateralis* (Olivier 1792) (M.L. Enríquez, S. Abril, M. Diaz and C. Gómez, unpubl.). Thus, in total, there were six 12x12m plots per location **(Figure 1)**. Nest searching in each plot was carried out by hand, lifting every single rock, branch or piece of debris, and gently moving the leaf litter with the help of a shovel at the bases of trees. Previous observations in the study area confirmed that the Argentine ant nests were not greatly disturbed by this sampling method, and that nests remained in place after several weeks. We marked active nests by a different coloured paint spray for each sampled period. The plots were checked exhaustively for nests every two months, and during a workday in each of the locations, from December 2008 to February 2010, and one additional monitoring in the following winter, December 2010. Nests were monitored from 9 a.m. to 3 p.m., when the ant activity is highest (Abril et al. 2007).

We categorized the locations by their Relative Integrated Anthropization Index (RIAI) (Martínez-Dueñas 2004) to evaluate the effect of the level of anthropization on the seasonal dynamics of the Argentine ant nests in each of the three locations sampled. We used the raster dataset showing land use classification in Catalonia 2002, 30x30 m, from LANDSAT-TM from the Department of the Environment (DMA), Government of Catalonia, with Miramon 7.0 GIS & RS software (Pons 2011).

2.2 Sampling methods

Seasonal variation in nests. To analyse seasonal variations in nests, plots were checked for active nests every two months during the first year of the survey, from December 2008 to December 2009. To carry out the statistical analysis, four periods were considered corresponding to the four seasons of the year.

Two-year extirpation effects. To analyse the effects of the two-year extirpation, we assessed the effects of the manual removal of winter nests, at the EX (extirpation) plots of each study area, in the winters of 2008/2009 and 2009/2010 - winter being the period of the year with the highest concentration of queens per nest (Abril et al. 2008b). We removed Argentine ant winter nests in January 2009 and January 2010, eliminating all of them by hand, digging the nests out with a shovel, and placing the soil removed into 25-litre plastic boxes previously smeared with Polytetrafluoroethylene on top to prevent ants from escaping **(Figure 3)**. Later in the laboratory we froze the boxes at -20ºC to kill individuals. To carry out the statistical analysis of the effects of the extirpation, we considered two periods of the year: pre-extirpation period (PRE, sampling before the manual removal of nests, previous December) and post-extirpation period (POST, sampling after the manual removal of nests, next late February).

Figure 3. Picture of a 25-litre plastic box smeared with Polytetrafluoroethylene and a shovel both used for placing Argentine ant winter nests to freeze and to kill individuals at -20ºC in the laboratory.

2.2.1 Nest and worker abundance

Five pitfall traps **(Figure 2)** were placed in fixed points and opened every two months for one week (from December 2008 to February 2010, and in December 2010), in order to estimate the worker abundance from each plot. Pitfalls were 5.5 cm diameter and 6.5 cm depth containing ethylene glycol (70%) to kill and to preserve individuals trapped. Abundance of nests was calculated as the sum of all nests found by plot each sampling period (every two months, from December 2008 to February 2010, and in December 2010).

2.2.2 Nest size and total area of nests per plot

We estimated nest size, also every two months (from December 2008 to February 2010, and in December 2010), following Heller (2004), dividing the study plot maps into 1 m grid squares and measuring the nest surface as the length of the longest diameter of the polygon multiplied by the length of the diameter orthogonal to the first. The total area of nests per plot was measured by adding all nests surfaces.

2.2.3 Spatial distribution of nests

We analysed the spatial distribution of nests every two months, from December 2008 to February 2010, and in December 2010, by mapping each of those found within each sampled plot, and using the Clark & Evans (1954) statistical test, R (ratio of the observed mean nearest neighbour distance (MNND) to the MNND expected for a randomly distributed population), from which values equal to 1 indicate random distribution, values significantly below 1 indicate aggregation and values significantly above 1 indicate overdispersion. We measured the distances between nearest neighbours from the map created, assuming nests within 40 cm or less to be the same nest, (Heller 2004).

2.2.4 Nest fidelity and nest lifetime

We evaluated changes in the number of active nests for each plot by means of two indexes: renewal index (RWI, the percentage of new nests that had appeared in a sample) and remaining index (RMI, the percentage of nests remaining from the previous sampling) (Cerdà et al. 2002). The nest lifetime was defined as the number of samples that remained each nest from opening to closure the whole sampling period (Cerdà et al. 2002, Heller & Gordon 2006), and was categorized using a scale of representative numbers: 1 - temporal (< 6 months); 2 - intermediate (6 ≤ months < 12); 3 - annual (12 months) and 4 - permanent (whole sampling period). We compared nest size with nest lifetime to find out whether larger nests remained active for longer periods.

2.3 Statistical Analysis

To evaluate the seasonal variation in nests during the first year, the effect of nest removal on the nest spatial distribution, and nest and worker abundance over the years sampled, we performed generalised linear mixed models (GLMMs). We used a Poisson error distribution with a log link function for nest and worker abundance, and for the persistence (RMI) and renewal of nests (RWI). In addition, we used a Gaussian error distribution with an identity link function to test for differences in the nest total area per plot, nest size and mean nearest neighbour distance (MNND). In regard to the seasonal variation of nests during the first year, nest and worker abundance, and nest total area per plot were evaluated using plot zone (contact, invaded or fully invaded) as fixed factor and location (CA, PD and PB) as a random factor. Persistence and renewal of nests were evaluated using plot type (CT or EX) as a fixed factor and location as a random factor. Nest size was evaluated using plot type (control or extirpation) and plot zone as fixed factors, and location as a random factor. To see whether the time that nests remained active depended on their size, we used nest size as fixed factor and location as random factor.

With respect to the two-year extirpation effects in the invasion front, we evaluated nest abundance and nest size using year (2008/2009 or 2009/2010) and period (PRE or

POST extirpation) as fixed factors, and location as a random factor. On the other hand, plot type (control or extirpation) and year were used as fixed factors, and location as a random factor to evaluate worker abundance. The mean nearest neighbour distance (MNND) were assessed with plot type and period as fixed factors, and location as a random factor, and renewal of nests with period as fixed factor, and location as a random factor. Finally, we evaluated whether larger nests remain active for a long time using nest size as fixed factor, and location as a random factor. We also used lineal regressions to evaluate the relationship between the time that nests remain active (nest lifetime) and their size, and a paired t-Student to evaluate nest size among seasons and to assign the spatial distribution of nests with the R index (Clark & Evans 1954).

All statistics were calculated using the R 2.12.1 for Windows package (R Development Core Team 2010) with a significance of $P < 0.05$. Variables were transformed in order to achieve normality in the linear regressions performed.

3. RESULTS

The level of anthropization in each of the three locations sampled was represented by the Relative Integrated Anthropization Index (RIAI). The locations (Santa Cristina d’Aro (CA), Pedralta (PD) and Puntabrava (PB)) presented the following values: $RIAI_{CA} = 27.87$, $RIAI_{PD} = 25.67$ and $RIAI_{PB} = 3.77$. The locations with the highest RIAI were Santa Cristina d’Aro and Pedralta, which means that they have a higher level of perturbation conducted by human presence than Puntabrava.

With respect to the two-year extirpation effects, plots in the Invaded (IN) and fully invaded (FIN) zones did not show differences in any of the variables studied, suggesting that nest removal was only effective at the edge of the invasion front, in the contact (CN) zone, where the population structure of the Argentine ant is weaker and more vulnerable. Then, further analyses regarding to the two-year extirpation effects were focused only at the invasion front.

3.1 Nest and worker abundance

Seasonal variation in nests. Nest abundance was higher at locations with a high Relative Integrated Anthropization Index (RIAI) (GLMM: P (|>χ^2|) = 0.02). These results suggest that nests in Santa Cristina d'Aro (the location with the highest level of anthropization) were more abundant **(Figure 4)**. In warmer seasons, when the biological activity in this Mediterranean zone is higher, workers of the Argentine ant were also more present than they were in winter (GLMM: P (|>χ^2|) < 0.001).

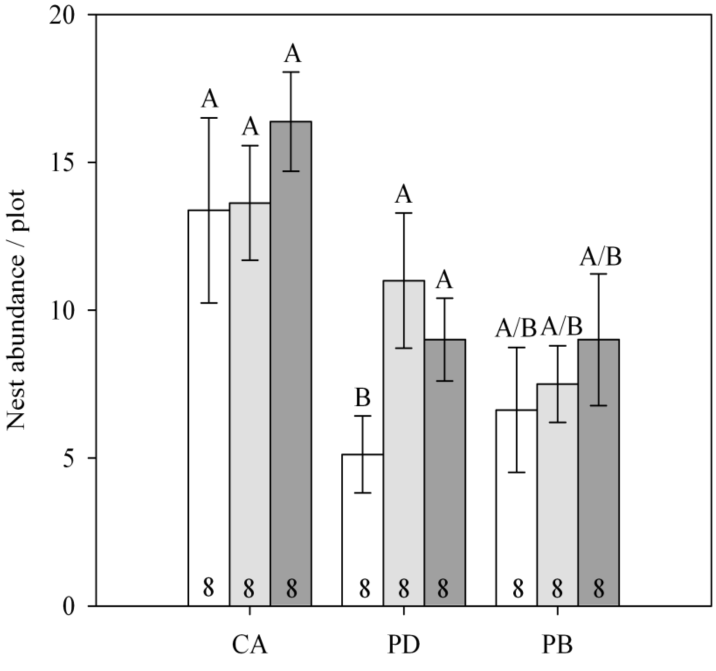


Figure 4. Nest abundance (mean ± SE) at each location (CA: Santa Cristina d'Aro, PD: Pedralta and PB: Puntabrava), and plot zone (i.e. distance from the invasion front): Contact (open bars), Invaded zone (light grey bars) and Fully invaded zone (dark grey bars). Sample sizes are shown inside bars. Letters indicate significant differences or similarities between nest abundance among plot zones.

Two-year extirpation effects. The mean number of nests also followed an annual fission-fusion cycle each year at control (CT) plots, being higher in POST period than in PRE period (GLMM: *P* ($|>\chi^2|$) = 0.03). On the contrary, at EX (extirpation) plots, the mean number of nests remained similar in both periods (GLMM: *P* ($|>\chi^2|$) = 0.43) **(Figure 5a).** The number of workers trapped by pitfalls before nest extirpation remained the same in the two first winters, but decreased in the third (GLMM: *P* ($|>\chi^2|$) <0.001). Although there were no significant differences in the number of workers between CT (control) and EX (extirpation) plots (GLMM: *P* ($|>\chi^2|$) = 0.22), in the first year, the CT (control) plots followed the annual cycle of contraction-dispersion, and in the second year the presence of workers were similar in both periods. This result could be a consequence of the increase of environmental temperature in the second year. In fact, workers at EX (extirpation) plots were less abundant the first year, and although the second year worker abundance was similar for both periods **(Figure 5b)**, it tended to decrease from PRE to POST extirpation periods. This result means that extirpation may have reduced the number of workers avoiding their dispersion in the period of nests fission.

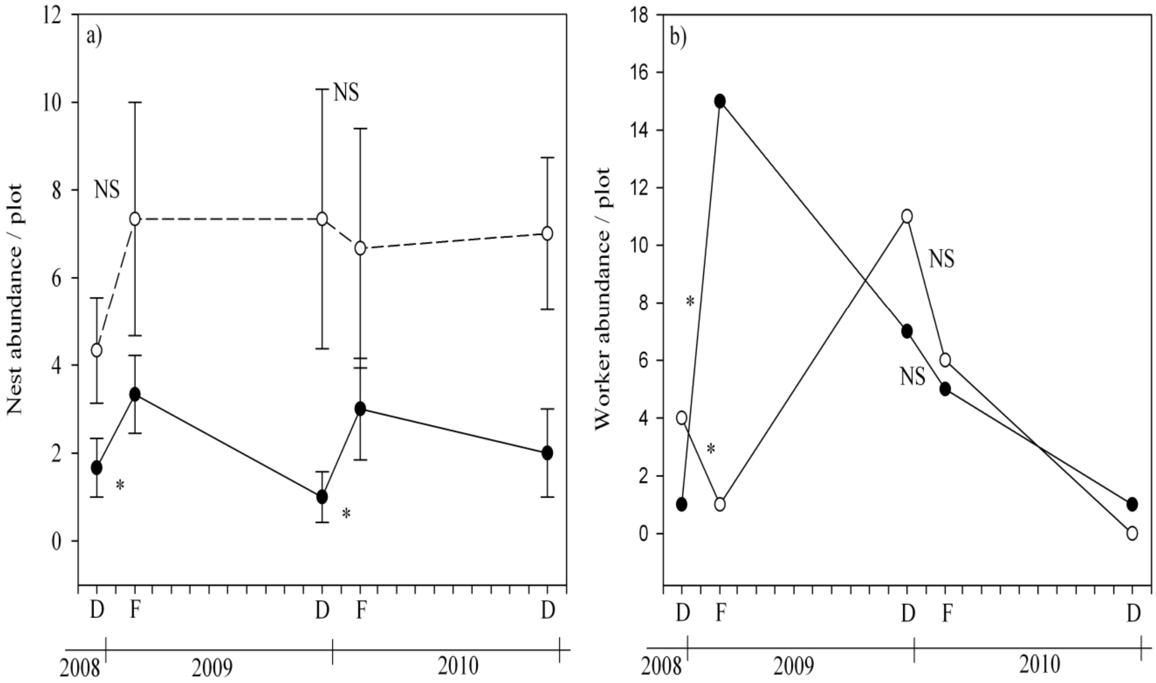


Figure 5. (a) Nest abundance (mean ± SE) and (b) worker abundance (total of individuals/plot) per plot (mean ± SE) time series in control (filled dots) and extirpation (open dots) plots. * $P < 0.05$, NS not significant. D: December (PRE-extirpation); F: February (POST-extirpation).

3.2 Nest size and total area of nests per plot

Seasonal variation in nests. Mean nest size depended on whether the plot was extirpated, therefore nests were bigger in the untreated control plots (GLMM: $F = 8.35$, $P = 0.005$) but also in plots further from the invasion front (GLMM: $F = 7.18$, $P = 0.001$), as well as the interaction between the two factors (GLMM: $F = 5.35$, $P = 0.007$). Nest size in untreated plots decreased as they came closer to the edge of the invasion front, while nests at treated plots remained the same regardless of the distance to the invasion front **(Figure 6a).** Seasonal climate variation also had an effect on nest size. We found larger nests in winter than in spring and summer (Paired t-Student winter/spring: $t = 2.92$, $P= 0.01$; winter/summer: $t = 3.87$, $P = 0.001$). In addition, the total area occupied by these nests per plot were higher at locations with a high Relative

Integrated Anthropization Index (RIAI) (GLMM: F = 5.18, P = 0.008), and at more invaded plots, where the invasion was better established (GLMM: F = 16.13, P < 0.001). **(Figure 6b).** These results suggest that nests were bigger and occupy a great extension of soil surface in Santa Cristina d'Aro (the location with the highest level of anthropization), and in invaded (IN) and fully invaded (FIN) plots.

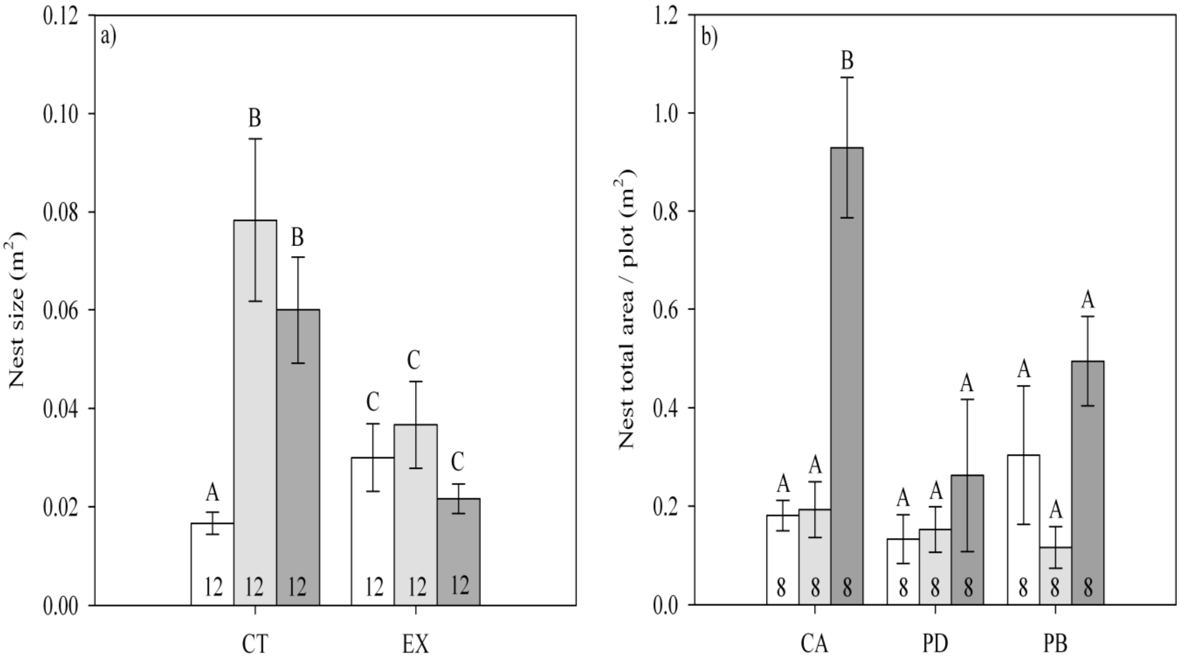


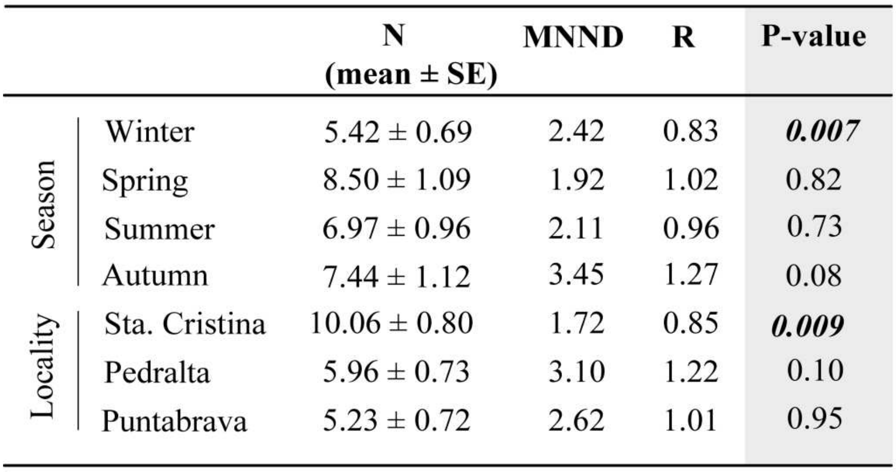
Figure 6. (a) Nest size (m^2) (mean ± SE) of control (CT) and extirpation (EX) plots in Contact (open bars), Invaded zone (light grey bars) and Fully invaded zone (dark grey bars). (b) Total area of nests per plot (mean ± SE) at each location (CA: Santa Cristina d'Aro, PD: Pedralta and PB: Puntabrava), and plot zone, i.e., distance from the invasion front: Contact (open bars), Invaded zone (light grey bars) and Fully invaded zone (dark grey bars). Sample sizes are shown inside bars. Letters indicate significant differences or similarities between abandoned or reoccupied nests among years.

Two-year extirpation effects. Nest size in extirpation plots (EX) remained similar over the two years and periods (PRE and POST extirpation). In contrast, although there were not differences in nest size (GLMM: F = 0.01, P = 0.9) between treatments and periods, nest size in CT (control) plots tended to decrease from winter to spring, founding larger nests in winter PRE period than in POST period.

3.3 Spatial distribution of nests

Seasonal variation in nests. Spatial distribution of nests was aggregated in winter at Santa Cristina d'Aro, the location with the highest RIAI. The rest of the seasons and locations, nests were randomly distributed **(Table 1)**.

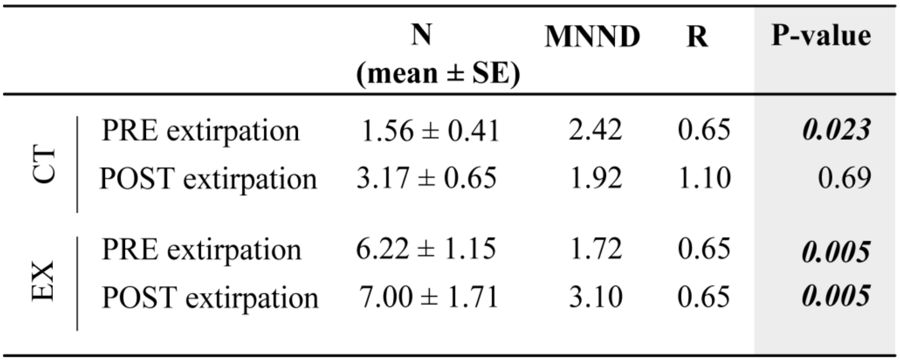
Table 1. Statistics of the number of nests per plot (N) and the mean nearest neighbour distance (MNND). R (coefficient Clark & Evans, 1954) significant differences of R to 1 (random distribution). *P-value* in bold italic is a significant value (*P < 0.05*).



		N (mean ± SE)	**MNND**	**R**	**P-value**
Season	Winter	5.42 ± 0.69	2.42	0.83	***0.007***
	Spring	8.50 ± 1.09	1.92	1.02	0.82
	Summer	6.97 ± 0.96	2.11	0.96	0.73
	Autumn	7.44 ± 1.12	3.45	1.27	0.08
Locality	Sta. Cristina	10.06 ± 0.80	1.72	0.85	***0.009***
	Pedralta	5.96 ± 0.73	3.10	1.22	0.10
	Puntabrava	5.23 ± 0.72	2.62	1.01	0.95

Two-year extirpation effects. The distribution of nests at non-treated plots followed the typical annual cycle, while at treated plots the nests were aggregated during the entire sampling. Statistics for mean nearest neighbour distance (MNND) are summarised in **Table 2**.

Table 2. Two-year extirpation statistics of the number of nests per plot (N) and the mean nearest neighbour distance (MNND). R (coefficient Clark & Evans, 1954) significant differences of R to 1 (random distribution). *P-value* in bold italic is a significant value (*P < 0.05*).



		N (mean ± SE)	MNND	R	P-value
CT	PRE extirpation	1.56 ± 0.41	2.42	0.65	***0.023***
	POST extirpation	3.17 ± 0.65	1.92	1.10	0.69
EX	PRE extirpation	6.22 ± 1.15	1.72	0.65	***0.005***
	POST extirpation	7.00 ± 1.71	3.10	0.65	***0.005***

The differences in MNND for the two periods (GLMM: *F* = 4.94, *P* = 0.042) and among different plot types (GLMM: *F* = 5.21, *P* = 0.037) were significant, as was the interaction between these two factors (GLMM: *F* = 6.02, *P* = 0.026). The mean nearest neighbour distance (MNND) rose at CT (control) plots from PRE to POST indicating the dispersal of nests following the temporal sequence of annual fission-fusion cycle, while for the EX (extirpation) plots, it remained the same in both periods **(Figure 7)**.

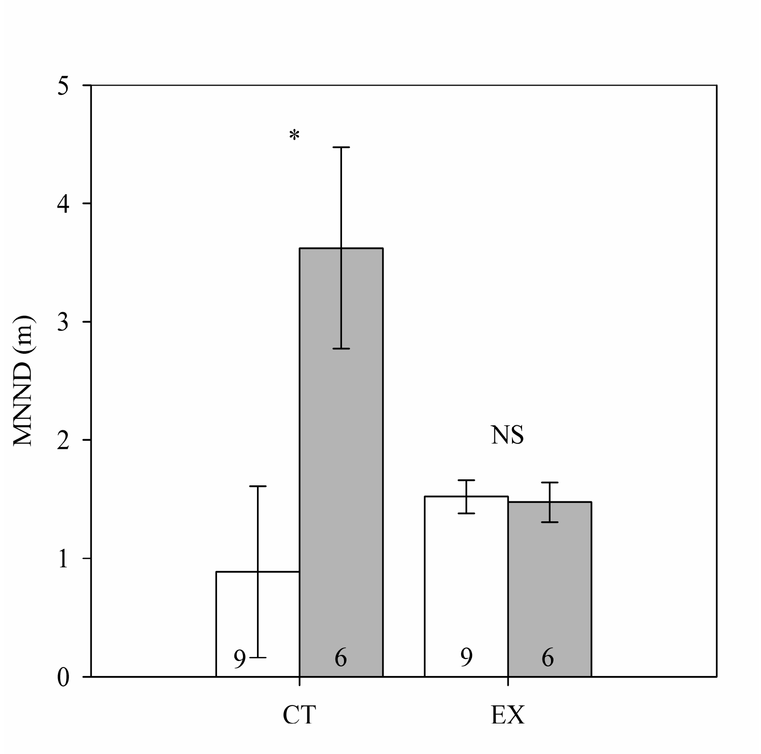


Figure 7. Mean nearest neighbour distance (MNND) (mean ± SE) in PRE-extirpation (open bars) and POST-extirpation (grey bars) periods, in control (CT) and extirpation (EX) plots. * $P < 0.05$, NS not significant. Sample sizes are shown inside bars.

Moreover, the highest MNND values were observed during the first year in POST period at CT (control) plots (4.30 ± 1 m SE), compared with the lowest values which were observed the same year in PRE period at CT (control) plots (0.35 ± 0.35 m SE). These results support the idea of a yearly seasonal cycle of nest distribution, with nest aggregation in winter and nest dispersal in spring.

3.4 Nest fidelity and nest lifetime

In total, in the three locations, we observed 585 nests, of which 81.2% remained active for less than six months, 15.7% for between 6 and 11 months, 2.6% for the whole first year, and 0.5% during the whole sampling period. The percentage of nests remaining (RMI) during the first year depends on whether the plot was extirpated (GLMM: $P\ (|>\chi^2|) < 0.001$). Therefore, nests remained for longer at non-treated plots than at treated ones (control/extirpation plots = 56.91 ± 5.29% / 40.69 ± 4.44%). It is also what happened at the contact zone (CN) for the two-year extirpation effects, where the RMI (remaining index of nests) was higher at CT plots with no removal of nests than at extirpation plots (GLMM: P $(|>\chi^2|) = 0.03$). However, the RWI (renewal index of nests) in this contact zone at CT (control) plots, were significantly different between periods (GLMM: P $(|>\chi^2|) = 0.002$), where renewal of nests happened only in spring. At the same time, in the EX (extirpation) plots the RWI was not significantly different (GLMM: P $(|>\chi^2|) = 0.69$), maintaining the same values for contraction and dispersion periods. Additionally, although there were no significant differences between treatments (GLMM: P $(|>\chi^2|) = 0.32$), the renewal of nests in the EX (extirpation) plots was 47% higher than among the CT (control) plots.

In addition, the time that nests remained occupied depends on their size ($r^2 = 0.22$, $P < 0.001$). Thus, larger nests remained occupied for longer time than smaller nests (GLMM: P $(|>\chi^2|) < 0.001$), and the mean nest size ± SE according to their lifetime was: temporal = 0.04 ± 0.01 m^2, intermediate = 0.29 ± 0.06 m^2, annual = 0.94 ± 0.29 m^2 and permanent = 0.16 ± 0.06 m^2.

The nests in the CT (control) plots that were constructed before extirpation (winter nests) tended to remain occupied for longer (GLMM: P $(|>\chi^2|) = 0.002$) because they were bigger than those constructed after extirpation (spring-summer nests) (GLMM: P $(|>\chi^2|) = 0.026$), while the nests in the EX (extirpation) plots were similar in size between periods (GLMM: P $(|>\chi^2|) = 0.56$), and in addition, a high percentage of them remained occupied for less time than those in non-perturbed plots. Nest size and nest lifetime decreased from one year to the next following a temporal sequence during the whole sample **(Figure 8).**

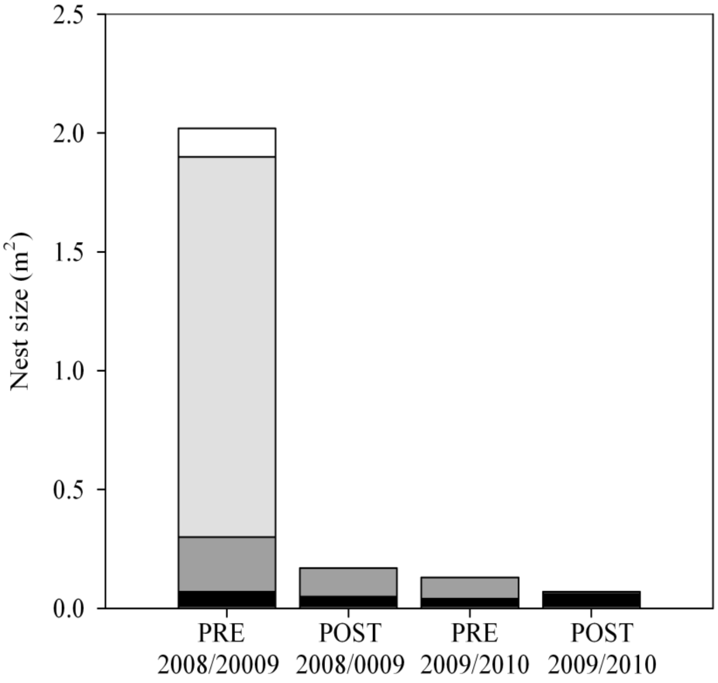


Figure 8. Nest size of nests (m^2) (mean ± SE) and nest lifetimes at extirpation plots: temporal (black stacks), intermediate (dark grey stacks), annual (light grey stacks) and permanent (open stacks) in each PRE and POST sample period of 2008/2009 and 2009/2010. PRE: pre-extirpation, POST: post-extirpation.

4. DISCUSSION

Results found on the seasonal variation in nest distribution support the idea of an annual cycle of fission-fusion pattern due to seasonal climate shown in previous studies (Benois 1973, Heller 2004). In winter, we found nests joining together to constitute “winter nests” in order to maintain optimal conditions for the Argentine ant activity. In spring and fall nests were randomly distributed over the plots having a dispersing period in spring and a contracting period in fall, when ants are looking for suitable places to nest in winter. Seasonal shifts in climate also influenced the activity and presence of workers. We found a higher abundance in warmer seasons, corresponding to their dispersal period and increase in their foraging activity (Abril et al. 2007, 2008b), than in winter, when their foraging activity is limited by the air temperature and limited to daylight hours (Abril et al.2007, Brightwell et al. 2010). In addition, our results agree with those found by Heller & Gordon (2006) in California, where nest surface area and abundance of nests in plots varied seasonally. Actually, nests observed in winter were larger but low in number, and in spring and summer they were smaller but more abundant.

Additionally, the interspecific competition exercised by native ants could have an influence on the presence of nests, the nest size and the nest total area that those occupied per plot. The Argentine ant may be had a lower level of interspecific aggression at locations and plots where it was better established and where there was a high RIAI (Relative Integrated Anthropization Index), such as in Santa Cristina d'Aro, and in the IN (invaded) or FIN (fully invaded) plots. In fact, in these areas *L. humile* has a stronger negative impact over the native ants, causing them to retract and therefore allow the Argentine ant to become the most abundant and dominant species in the community (Suarez et al. 2001, M.L. Enríquez, S. Abril, M. Diaz & C. Gómez, unpubl.). Consequently, the pressure the Argentine ant is subject to is low, allowing their nests to increase in number and grow in extension. On the contrary, at contact plots close to the edge of the invasion front, where the native ant community is more structured, this invasive species is subject to a higher interspecific competition for new nesting sites and food resources (Ingram 2002a, Sanders et al. 2003). In these plots the Argentine ant nests are limited in number and size. Also we could consider that this gradual decreasing in nests abundance and nest size in part is due to the result from the history of the Argentine ant colonies dispersion. In fact, colonies closer to the invasion front should be smaller than those found in the invaded or fully invaded plots because they result from recent dispersion of smaller propagules.

On the other hand, manual removal of nests carried out in January 2009 produced changes in nest size. Due to the perturbation they were subjected, nests in the EX (extirpation) plots were smaller and of a similar size in each plot regardless of the distance from the invasion front. In contrast, nests in the CT (control) plots were larger and followed the typical seasonal cycle of contraction-dispersal, and decreased the closer they were to the edge of the invasion front. As a matter of fact, the closer we get to the invasion front, the better structured the local ant community is, and thus interspecific interactions occur more frequently, forcing the Argentine ant to compete more strongly for new nesting sites and resources (Ingram 2002b) in these areas.

Like Heller & Gordon (2006) in California, we also observed that larger nests, which were mostly found in winter, were more likely to persist throughout the cold season than smaller nests, which were usually found in late spring or summer. A 2.6% of larger winter nests persisted the whole year, suggesting that, as proposed by Heller et al. (2008a), they are mature nests that act as mother nests providing a source of queens for the colonization of new non-invaded areas in the expansion period of this species. Moreover, these nests were found in each plot along the edge of the invasion front, but mature old nests found in the invaded zones (IN and FIN) were bigger than those found in the contact (CN) zone. Perhaps these bigger and more persistent nests in the contact zones were still forming as mother nests and had not achieved the maturity gained by those nests in the invaded zone where they had been active for much longer. However, compared to the Heller & Gordon (2006) results, we found a higher proportion of temporal (80%) and smaller (70%) nests. Additionally, they were more abundant (5% more) in extirpation plots compared to control plots. The increase of temporal nests in extirpated plots could be a consequence of the extirpations done in January 2009 and January 2010, which divided those mature nests and made them unstable and less persistent, forcing them to move and find other suitable sites. Therefore, these nests became similar to those found in summer, which do not remain in the same place for a long time.

The two-year management by means of manual removal of nests in winter would appear to be effective only at the edge of the invasion front where the Argentine ant population is less structured, weaker and more vulnerable to disturbance. Extirpation at the edge of the invasion front promotes changes in spatial distribution and the number and size of nests, as well as in the time that nests remain active over the sampling period of this study, and also on the abundance of workers. Number and size of nests per plot in the EX (extirpation) plots were similar and they remained aggregated in both periods, before and after the removal of winter nests. In contrast, undisturbed plots followed the typical cycle of larger, less abundant and contracted nests in winter (PRE period), and smaller, more abundant and dispersed nests in early spring (POST period). Nests at CT (control) plots increased an average of 57.8% from winter to spring during the two first years, while at treated plots nests remained in the same level of abundance. In total, at non-extirpated plots nests rose a 16.7% from the beginning of the experiment. This would mean that without a management of the Argentine ant invasion, it would expand its range each year. Therefore, the manual removal of nests in winter may reduce the expansion of the invasion during the early dispersing period of nests. An explanation for this result could be that queen and worker elimination slows the dispersal capacity of the colony, as the manual removal reduces the number of spreading gynes and individuals. Another explanation could be that after the extirpation the ants invest more energy to reconstruct the original perturbed nest rather than building up new nests, this worker priority might delay the dispersal of the colony. Moreover, the reduced presence of workers found at EX (extirpation) plots after removal of nests could be another factor that affected the expansion.

The two first winters, plots disturbed by manual removal experienced a decrease in workers after the treatment. It was observed that at control plots the number of ants augmented from winter 2008/09 to spring 2009 because of the dispersion, while from winter 2009/10 to spring 2010 the presence of workers diminished. This was probably a consequence of the late snowfalls at the beginning of March 2010, which did not allow foraging activity by this species until late spring, after we had taken spring samples. Although over the two-year extirpation we still have no significant results yet, we have seen a decreased tendency in the number of nests and workers observed at the treated plots from winter to spring during the two years of extirpation, but they recovered again in December 2010.

Summarizing, during the first year (December 2008-December 2009) all variables tended to follow an annual cycle of contraction and dispersal. The number of nests decreased the closer they were to the edge of the invasion front, possibly due to competition with native ants. Extirpation decreased the size of nests and the time they remained active during the sampling period.

The two-year extirpation promoted smaller and aggregated nests and a decrease in the number of workers per plot during the first and second year of the study. Moreover, although number of nests remained similar between periods, there seemed to be a tendency for this number to decrease between December 2008 and December 2009, and to recover in December 2010. This means that to have significant results and to keep the number of nests, and in consequence the expansion of the Argentine ant within a low rate, a yearly extirpation will be required. Together with extirpation other biological control methods could be integrated, like foraging disruption (Nisishue et al. 2009) or diet dissociation (Silverman & Liang 2001, Guerrieri et al. 2009) to decrease the invasive power of this species at the edge of the invasion front.

CHAPTER 4. Is the submissive ant *Plagiolepis pygmaea* affected by the presence of the Argentine ant in natural invaded areas?

(picture: Nikola Rahmé)

Is the submissive ant *Plagiolepis pygmaea* affected by the presence of the Argentine ant in natural invaded areas?

1. INTRODUCTION

The Argentine ant (*Linepithema humile*, Mayr 1868) is a well-known invader in Mediterranean-type climates. In the Iberian Peninsula, this ant species is present along the coastline, including areas of natural interest (Espadaler & Gómez 2003) such as secondary open cork oak forests on the southern edge of the Gavarres and Cadiretes massifs. The numerical dominance of the Argentine ant over native ants influences the outcome of competitive interactions among them and the native species (Heller et al. 2008b), and causes the displacement of the latter (Human & Gordon 1997, Holway 1998b, Gómez & Oliveras 2003).

The Argentine ant is heavily influenced by temperature and water availability. Temperature has a strong influence on some of the species' reproductive traits (Newell & Barber 1913, Benois 1973, Abril et al. 2008a) and on foraging effectiveness (Markin 1970, Human & Gordon 1999, Witt & Giliomee 1999, Holway et al. 2002a, Abril et al. 2007, Jumbam et al. 2008). Additionally, water availability determines the abundance and distribution of the species in Mediterranean-type systems (Human & Gordon 1999, Suarez et al. 2001, Holway et al. 2002a, Jumbam et al. 2008) and plays an important role in 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 hot and dry Mediterranean environments (Schilman et 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 et al. 2002a, Abril et al. 2007) and why low soil moisture limits its expansion (Holway et al. 2002a, Menke & Holway 2006, Menke et al. 2007).

In these Mediterranean ecosystems, environmental factors show important seasonal variations and the limited thermal tolerance of dominant species towards subordinate species could disrupt the expected transitive hierarchies. Consequently, these variations allow a more substantial presence of the subordinate species in the ecosystem (Cerdà et al. 1997). One such subordinate species present in the invaded area that has been frequently associated with the Argentine ant is *Plagiolepis pygmaea* (Latrielle 1798) (Oliveras et al. 2005, Gómez & Espadaler 2006, Abril & Gómez 2009, Roura-Pascual et al. 2010). It seems that the ability to avoid confrontation and the use of extremely submissive behaviour to appease opponents promotes the lack of aggression of the Argentine ant towards it, contributing to the coexistence of the two ant species (Abril & Gómez 2009). However, their coexistence cannot be totally explained as a result of a habituation process (Abril & Gómez 2009), as was reported in the case of the coexistence between *Wasmannia auropunctata* and the native *Cyphomyrmex genus* in French Guiana (Grangier et al. 2007).

In previous studies (**CHAPTER 1**), we observed an interesting fact: it seemed that in spring-summer *P. pygmaea* had occupied the abandoned winter nests of the Argentine ant. *P. pygmaea* and the Argentine ant have a similar colony structure. Both species are highly polygynous (several queens per colony) and form large polydomous colonies comprising several nests between which queens, workers, and brood are exchanged (Passera 1963, Heller & Gordon 2006, Abril et al. 2008b). Additionally, the number of nests per colony varies seasonally. In spring colonies of both species split into numerous nests, which remain in close proximity to one another (Passera et al. 2001, Heller et al. 2006). *P. pygmaea* in late summer and *L. humile* in late fall fuse the nests with other colony subunits in large formations known as “winter nests”, thus reducing the level of polydomy for overwintering (Newell & Barber 1913, Passera et al. 2001). These similarities in colony structure and the continuous movement of both species’ nests lead us to believe that like the Argentine ant, *P. pygmaea* probably makes little effort to build up its nests (Newell & Barber 1913, Markin 1970, Heller 2004, Heller & Gordon 2006), leading the latter to take advantage of the abandoned Argentine ant winter nests in spring-summer and vice versa.

Nevertheless, little is known about the exchange of nests between *P. pygmaea* and the Argentine ant. Taking this into account, the purpose of this study is to determine the factors that promote this exchange of nesting sites between the Argentine ant and *P. pygmaea*, and ascertain whether the latter could take advantage of the invasion in terms of resource competition. To achieve this, for both species we specifically (1) examined the dynamic populations of nests in terms of nest density, worker abundance, and nest exchange, and (2) characterized the abiotic components (physical characteristics, temperature, and water availability) of nests.

2. MATERIALS AND METHODS

2.1 Study area

We studied nests of both *Plagiolepis pygmaea* and the Argentine ant from September 2010 to May 2011 at three invaded localities of open cork oak secondary forests. One locality was placed on the southern edge of the Gavarres Massif in the area of Santa Cristina d'Aro (CA, 41°48'51.71"N 3°01'50.57"E), and two in the Cadiretes massif in the areas of Pedralta (PD, 41°47'31.53"N 2°58'52.79"E) and Puntabrava (PB, 41°46'13.51"N 3º00'17.93"E), in the northeast of the Iberian Peninsula **(Figure 1).** These three zones were at least 7 km apart from each other. This region has a

Mediterranean climate with a mean annual rainfall of 690 mm and a mean annual temperature of 15.5ºC.

2.2 Dynamic populations: Nest density, worker abundance and nest exchange of *P. pygmaea* and *L. humile*

From September 2010 to May 2011 we evaluated both the spatial distribution of nests and the *P. pygmaea* and *L. humile* nest exchange dynamics. To estimate nest density and nest exchange for both species, we searched for *P. pygmaea* and *L. humile* nests in two zones of each locality (CA, PD and PB): native zone (nests located in front of the edge of the invasion at a mean of 130 m, with the presence of native ant species only), and invaded-front (nests located behind the edge of the invasion at a mean of 20 m, in contact with native ant species) **(Figure 1)**.

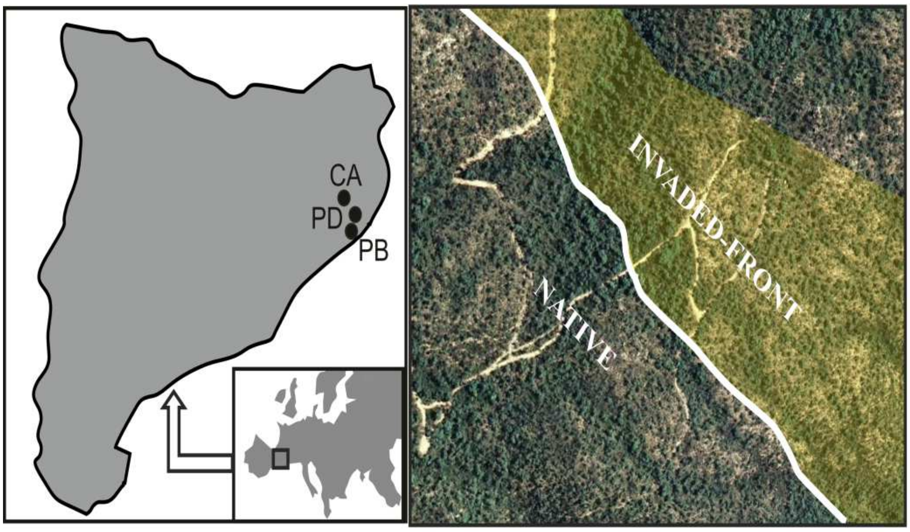


Figure 1. Areas invaded by the Argentine ant colonies in the northeast of the Iberian Peninsula. Black circles indicate localities sampled (CA: Santa Cristina d'Aro, PD: Pedralta and PB: Puntabrava). The map on the right illustrates the front of the invasion (white line) and the two areas sampled (native and invaded-front) of PB. (source: Google^{TM} earth 2012)

In order to determine changes in nest density and the exchange of nests between the two species under study, we searched for nests for a period of three hours in each zone and locality, lifting every rock carefully to disturb the nest as little as possible. Nests found belonging to both ants were marked with a different-coloured spray paint to identify them as one or the other species. Nests were checked every two weeks, and monitored for activity. The presence of new *P. pygmaea* and *L. humile* nests discovered within a radius of 2 m from the primary nests found was also recorded. Nests were categorized as (1) active nests (nests with workers present on the top and/or registering some visible activity), (2) non-active nests (nests without the presence of workers in the top and/or registering a lack of visible activity), (3) renewed nests (nests from the previous sampling reactivated with the presence of workers in the top and/or registering some activity inside after overwintering) and (4) newly built nests (new nest to appear in a sampling). Additionally, we noted the number of nests that had registered an exchange in their guest species. Nest density was calculated as the presence of nests of each species per sampled area of each zone and locality.

We also included control points for each *P. pygmaea* and *L. humile* nests, free of ants, to compare environmental factors (i.e. temperature and soil water content) inside and outside the *P. pygmaea* and *L. humile* nests. Each of these control points was located in a random direction 2 m away from each sampled nest.

To estimate worker abundance for both species, we used data from **CHAPTER 3,** where the study of worker abundance for *P. pygmaea* and *L. humile* was estimated by locating five pitfall traps (from August to June in a total of six 12x12 m plots) and sampling a total of two plots by locality studied**.** One plot was located in the native zone and the second plot was located in the invaded-front**.** Pitfalls were 5.5 cm in diameter and 6.5 cm deep, and contained ethylene glycol (70%) in order to kill and preserve individuals trapped. Pitfalls were placed at fixed points along the study and opened every two months for one week **(Figure 2, CHAPTER 3)**.

2.3 Nesting site characterization: physical characteristics, temperature and soil water content

To determine the physical characteristics of both *P. pygmaea* and *L. humile* nests we 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), and distance to the nearest tree (m). In addition, we registered the distance to the nearest nest of the same species for both *P. pygmaea* and *L. humile*, and the distance to the nearest nest of the other species. We recorded the location of each nest using a Garmin eTrex Legend® HCx GPS with an accuracy of 3 m, as well as orientation and distance to the nearest nest (calculating the Euclidean distances with Mapsource_v6.13.7 Extreme GPS software). Finally, we measured the canopy cover with digital photographs of the coverage at each site. We analysed the pictures with GapLightAnalyzer_v2 software, which estimates the percentage of canopy openness. We used this to calculate the percentage of canopy cover as: 100 - canopy openness %.

We also measured the soil temperature (ºC) and soil volumetric water content (VWC, %) of each nest and their respective control points. We took temperature measurements by means of HOBO ® H8 Pro Series data loggers from 9 a.m. to 3 p.m. on the day of the survey, placing the external sensor of the data logger 5 cm below the surface of the soil. At each nest and its respective control point we also measured the soil VWC three times on the day of the survey (at 9 a.m., 12 p.m., and 3 p.m.) using a sensor Field Scout TDR 100/200, which measured the VWC across the surface and to a depth of up to 12 cm in the soil.

2.4 Data analysis

We made all the comparisons between nest density, worker abundance, nest exchange, canopy cover, and water content from different zones, species, and nest activity using generalized linear mixed models (GLMMs) with a Poisson error distribution and logit link function. Distance to the nearest tree, nearest nest of the same species, nearest nest of a different species, and temperature were assessed using GLMMs with Gaussian error distribution and an identity link function. In comparisons between zones we used locality (Santa Cristina d'Aro, Pedralta, or Puntabrava) as a random factor, and the zone (native or invaded-front) and the season as fixed factors. In comparisons between species we used locality as a random factor, and the species (*P. pygmaea* or *L. humile*) and the season as fixed factors. In comparisons between active and non-active nests of *P. pygmaea* we used nest activity and season as fixed factors, and locality as a random factor. In the case of orientation between zones and species, we ran a multinomial logistic regression (MLR) with the season and zone or species as fixed factors. We also compared temperature and VWC of *P. pygmaea* nests and their respective control points using sample type (nest or control) as a fixed factor and nest identity as a random factor. All statistics were calculated using the R 2.12.1 for Windows package (R Development Core Team 2010) for all analyses.

3. RESULTS

3.1 Dynamic populations: nest density, worker abundance and nest exchange of *P. pygmaea* and *L. humile*

In the native zone nest density of *P. pygmaea* followed the typical seasonal dynamic found by Passera (1994). Worker abundance also concurred with the normal seasonal polydomy shown by this species **(Figure 3)**.

In the invaded-front *P. pygmaea* displayed behaviour similar to that of the native zone, with a period of declining activity in winter. However, it seemed that in this period of hibernation *P. pygmaea* still had any visible activity inside nests in the invaded-front. On the other hand, *L. humile* also fit with the typical seasonal cycle, reducing its activity in winter, but with the known lack of hibernation period for this species (Newell & Barber 1913, Markin 1970, Benois 1973, Heller & Gordon 2006, Abril et al. 2008b) **(Figure 3)**. Then, *P. pygmaea* nest density percentage and worker abundance were significantly different between zones and among seasons (GLMM, nest density: P (|>χ^2|) <0.001; worker abundance: P (|>χ^2|) = 0.03) **(Figure 3)**.

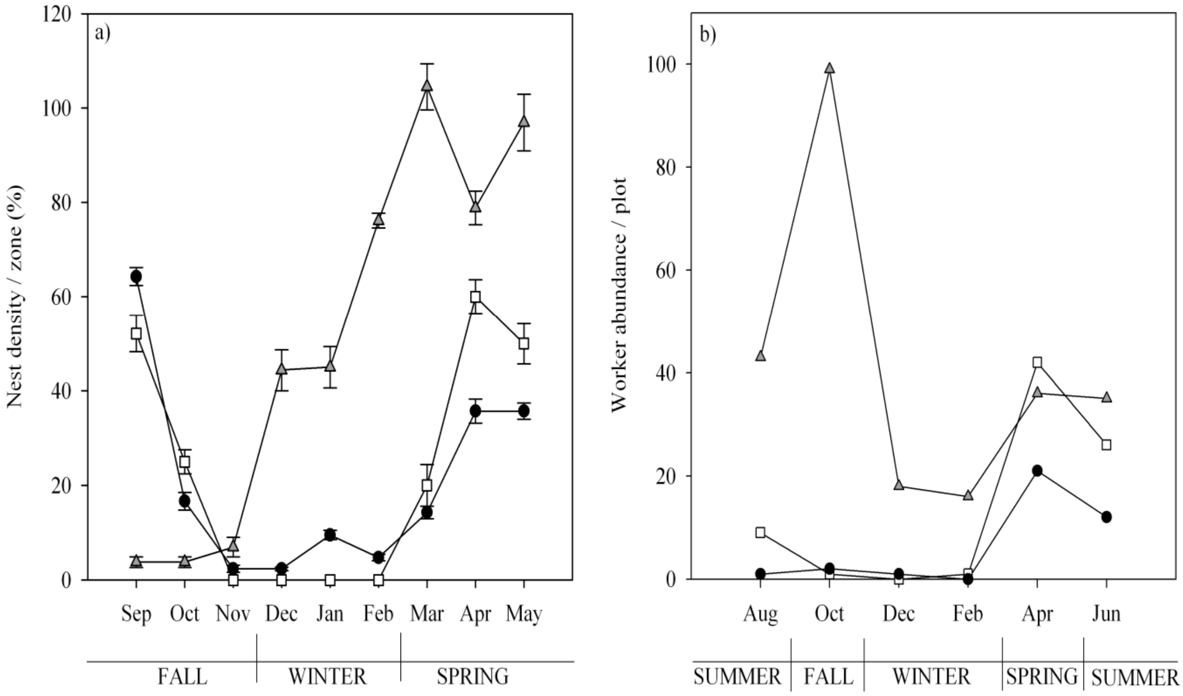


Figure 3. (a) Nest density (%) (mean ± SE)and (b) worker abundance (total of individuals/plot) of *P. pygmaea* in the native zone (open squares), *P. pygmaea* in the invaded-front (fill dots) and *L. humile* in the invaded-front (grey triangles).

On the other hand, *P. pygmaea* active nests, non-active nests, and renewed nests in spring in the native area are shown in **Figure 4a**. *P. pygmaea* renewed its nest in spring in the same place in a mean of 26.3 ± 4.9% (SE) and colonized new nesting sites in a mean of 18.15 ± 1.35% of cases. The peak of maximum activity for *P. pygmaea* in the native zone was in April, when the maximum renewal of nests and the maximum newly built nests were registered.

Additionally, active nests, non-active nests, and nests renewed for both species in the invaded-front are shown in **Figure 4b and 4c**. *P. pygmaea* colonized new nesting sites more frequently (4.84 ± 0.92%) than it renewed previously founded nests (26.43 ± 4.39%) in spring. The peak of maximum activity of *P. pygmaea* in the invaded-front was in April, when the maximum renewal of nests took place, whereas May saw the maximum newly built nests. Moreover, in November and until late spring, the Argentine ant began to occupy the *P. pygmaea* winter nests that had been without activity. The Argentine ant occupied more nests of *P. pygmaea* without any activity in winter (83.3 ± 16.67%) than in spring (76 ± 10.3%). Additionally, nests of *P. pygmaea* with activity were occupied by *L. humile* only in one case in winter, but in 24.07 ± 10.3% of cases in spring. On the other hand, in late winter and in spring *P. pygmaea* occupied some old abandoned nests of *L. humile*, and in no instance would those nests be subsequently occupied by the Argentine ant.

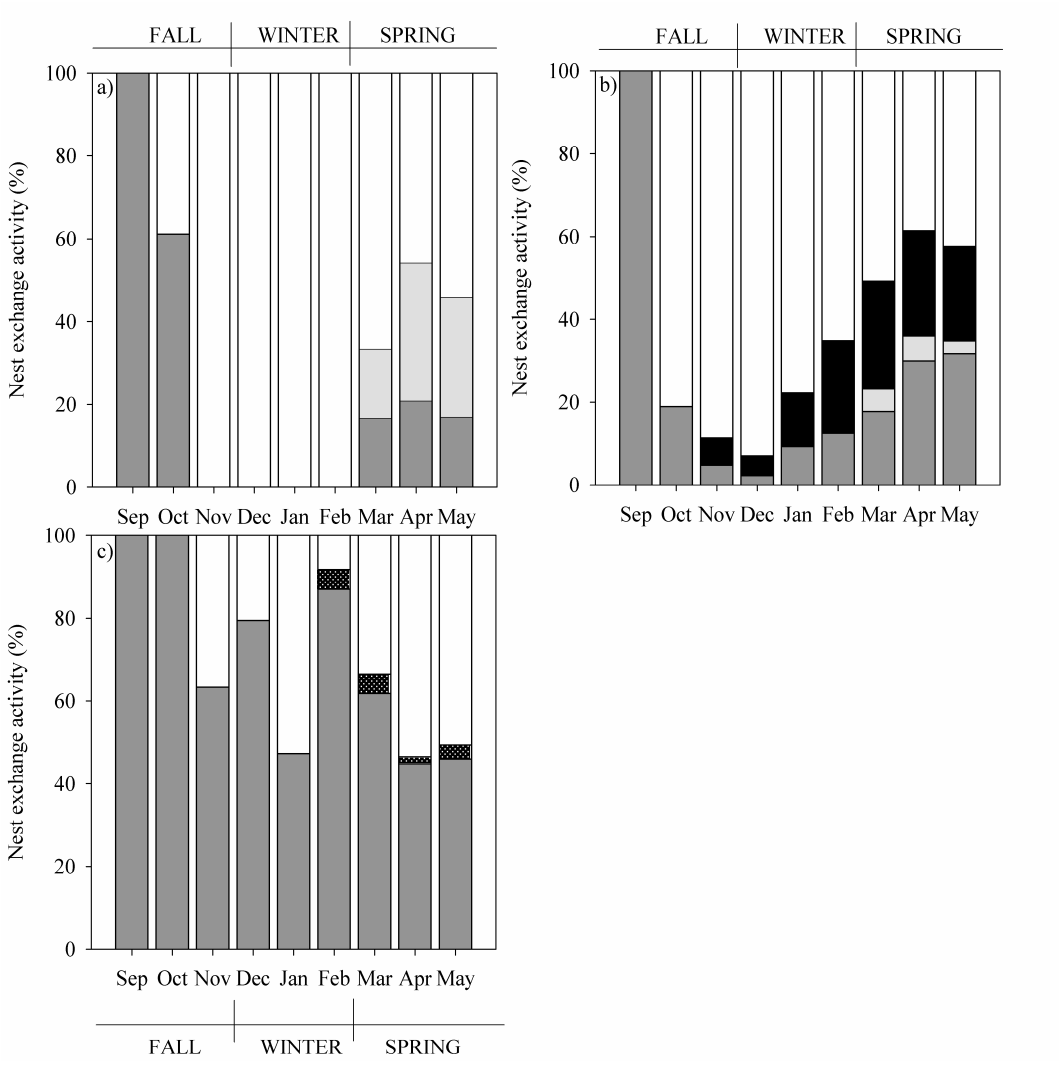


Figure 4. Percentage of P. *pygmaea* active nests (dark grey stacks), non-active nests (open stacks) and renewed nests (light grey stacks), and nests of *P. pygmaea* occupied by *L. humile* (black stacks) (a) in the native zone and (b) in the invaded-front; (c) Percentage of *L. humile* active nests (dark grey stacks), non-active nests (open stacks) and nests of *L. humile* occupied by *P. pygmaea* (black dotted stacks) in the invaded-front.

The number of *P. pygmaea* active nests and newly built nests were statistically different when compared across zones and seasons (GLMM, active nests: P (|>χ^2|) < 0.001 and newly built nests: P (|>χ^2|) < 0.001), while the number of renewed nests were significantly different for each zone (GLMM: P (|>χ^2|) < 0.001) and season (GLMM: P (|>χ^2|) < 0.001) separately, but not for the interaction between these two factors (GLMM: P (|>χ^2|) = 0.6), indicating that only in spring there was a renewal of *P.*

pygmaea nests. Nest exchange activity was higher in the native zone in spring and higher in the invaded-front in winter, when the species is inactive due to its hibernation period **(Figure 5)**.

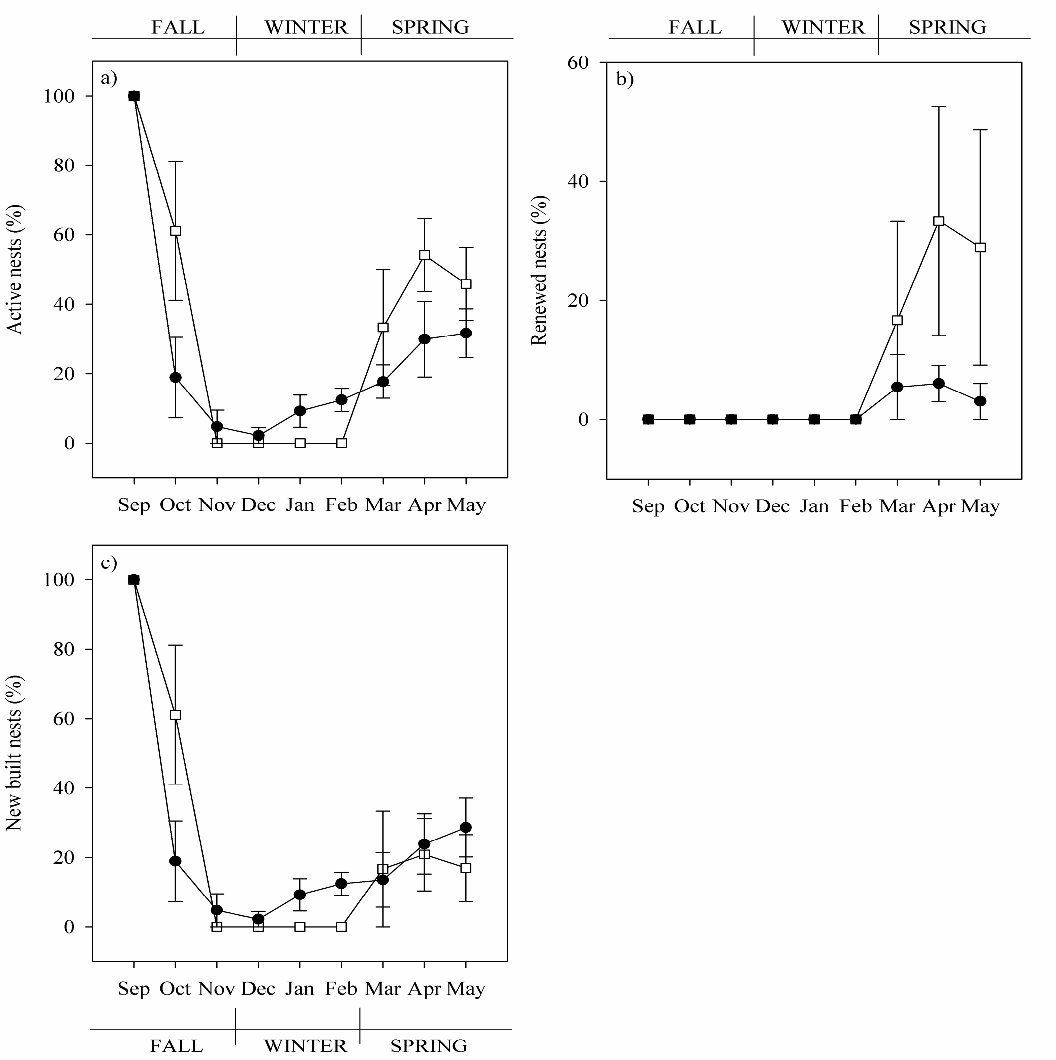
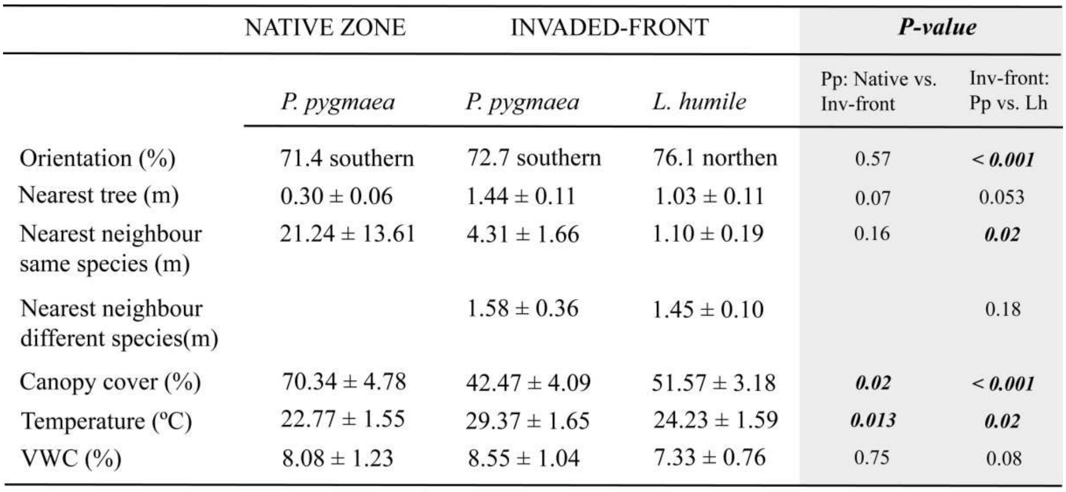


Figure 5. (a) Active nests (%), (b) renewed nests (%) and (c) newly built nests (%) (mean ± SE) of *P. pygmaea* in the native (open squares) and in the invaded-front (filled dots).

3.2 Nesting site characterization: physical characteristics, temperature and soil water content

Physical characteristics of *P. pygmaea* and *L. humile* nests in the native and the invaded-front are shown in **Table 1** and **Figure 6**.

Table 1. Characteristics of *P. pygmaea* and *L. humile* nests (mean ± SE) in the native and the invaded-front. *P-value* in bold italic is a significant value ($P < 0.05$). Inv-front: invaded-front, Pp: *P. pygmaea*, Lh: *L. humile*.



	NATIVE ZONE	INVADED-FRONT		***P-value***	
	P. pygmaea	*P. pygmaea*	*L. humile*	Pp: Native vs. Inv-front	Inv-front: Pp vs. Lh
Orientation (%)	71.4 southern	72.7 southern	76.1 northen	0.57	***< 0.001***
Nearest tree (m)	0.30 ± 0.06	1.44 ± 0.11	1.03 ± 0.11	0.07	0.053
Nearest neighbour same species (m)	21.24 ± 13.61	4.31 ± 1.66	1.10 ± 0.19	0.16	***0.02***
Nearest neighbour different species(m)		1.58 ± 0.36	1.45 ± 0.10		0.18
Canopy cover (%)	70.34 ± 4.78	42.47 ± 4.09	51.57 ± 3.18	***0.02***	***< 0.001***
Temperature (ºC)	22.77 ± 1.55	29.37 ± 1.65	24.23 ± 1.59	***0.013***	***0.02***
VWC (%)	8.08 ± 1.23	8.55 ± 1.04	7.33 ± 0.76	0.75	0.08

Canopy cover above *P. pygmaea* nests, and distance to the nearest tree were different between zones and seasons. However, orientation and nearest nest distance were the same in both zones and same seasons. During the cold season, nests were mainly southerly facing and more aggregated, while in warm season nests were mainly northerly facing and more dispersed. On the other hand, canopy cover was different between species (*L. humile* and *P. pygmaea*) and seasons in the invaded-front. In colder seasons canopy cover was higher for *L. humile,* and in warmer seasons it was the same for both species. Although distance to the nearest tree was not significantly different between species, it was a little bit higher for *P. pygmaea* during spring. Distance to the nearest nest of the same species was significantly different between *P. pygmaea* and *L. humile,* being higher for *P. pygmaea* in colder seasons, while in spring it was higher for *L. humile*. The distance to the nearest nest of a different species was not significantly different between both species and seasons.

In regard to orientation, both species faced different sides in colder seasons while in warmer seasons they faced the same side. Nests of *P. pygmaea* were mainly southerly facing in fall and winter, while in spring they were northerly facing. In contrast, nests of *L. humile* were mainly northerly facing in each season.

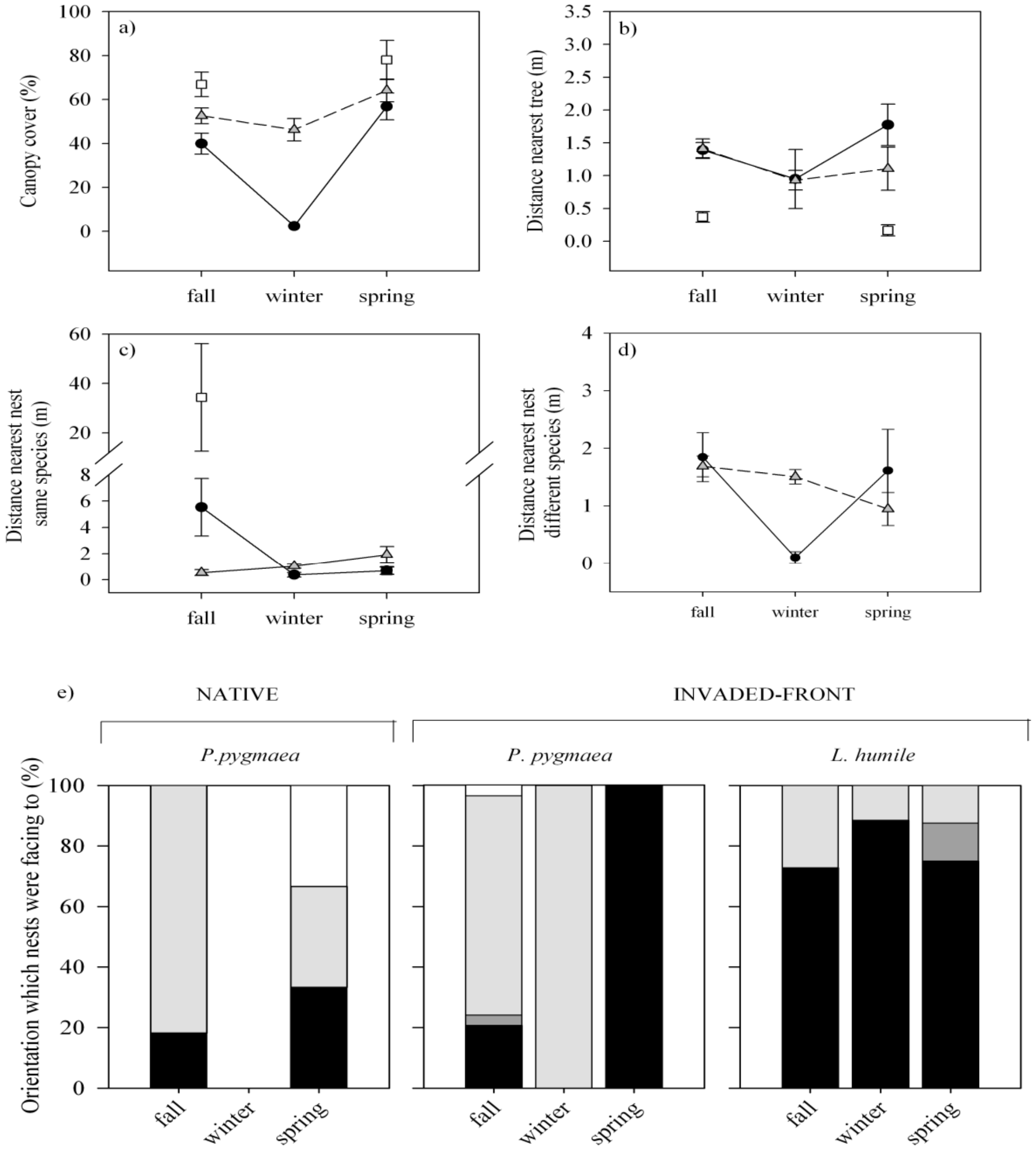


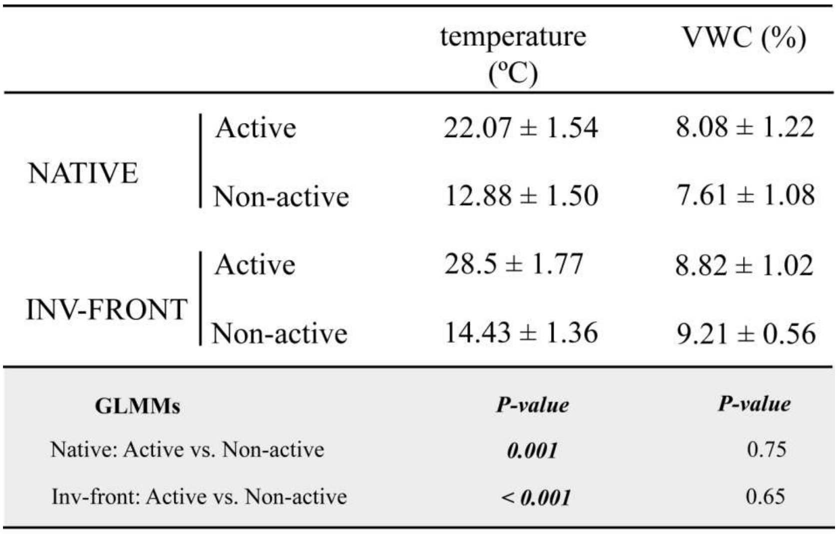
Figure 6. Physical characteristics of *P. pygmaea* nests (mean ± SE) in the native (open squares), and *P. pygmaea* (filled dots) and *L. humile* nests (grey triangles) in the invaded-front during the sampled period. Nest orientation was categorized as: North (black stacks), East (dark grey stacks), South (light grey stacks) and West (open stacks).

Comparisons in temperature of *P. pygmaea* active nests between the two zones (native and invaded-front) were made during spring only, as nest activity in the native zone was null in winter. Temperatures in spring, however, were significantly different between zones, as *P. pygmaea* in the invaded-front tended to nest in warmer areas than in the native one. In contrast, the VWC was the same in both zones.

On the other hand, temperature and VWC between *P. pygmaea* nests and their control points in both native and invaded-front were the same (GLMM, all $P > 0.05$). These variables only show differences over time, due to shifts in environmental factors during the sample period. However, active and non-active *P. pygmaea* nests in both the native and the invaded-front presented differences in temperature, the active nests being warmer than the non-active ones (**Table 2**, **Figure 7a** and **7b)**.

Non-active nests were present mainly in winter, while in spring they were only present in the invaded-front. Regarding VWC, active nests and non-active nests were the same throughout the sample period in both zones **(Table 2, Figure 7c** and **7d),** and the nests always remained within the same range.

Table 2. Temperature (ºC) and VWC (%) (mean ± SE) of *P. pygmaea* active and non-active nests in both the native and the invaded-front. GLMMs (Generalised Linear Mixed Models) *P-value* in bold italic is a significant value (*P < 0.05*). Inv-front: invaded-front.



		temperature (ºC)	VWC (%)
NATIVE	Active	22.07 ± 1.54	8.08 ± 1.22
	Non-active	12.88 ± 1.50	7.61 ± 1.08
INV-FRONT	Active	28.5 ± 1.77	8.82 ± 1.02
	Non-active	14.43 ± 1.36	9.21 ± 0.56

GLMMs	***P-value***	***P-value***
Native: Active vs. Non-active	***0.001***	0.75
Inv-front: Active vs. Non-active	***< 0.001***	0.65

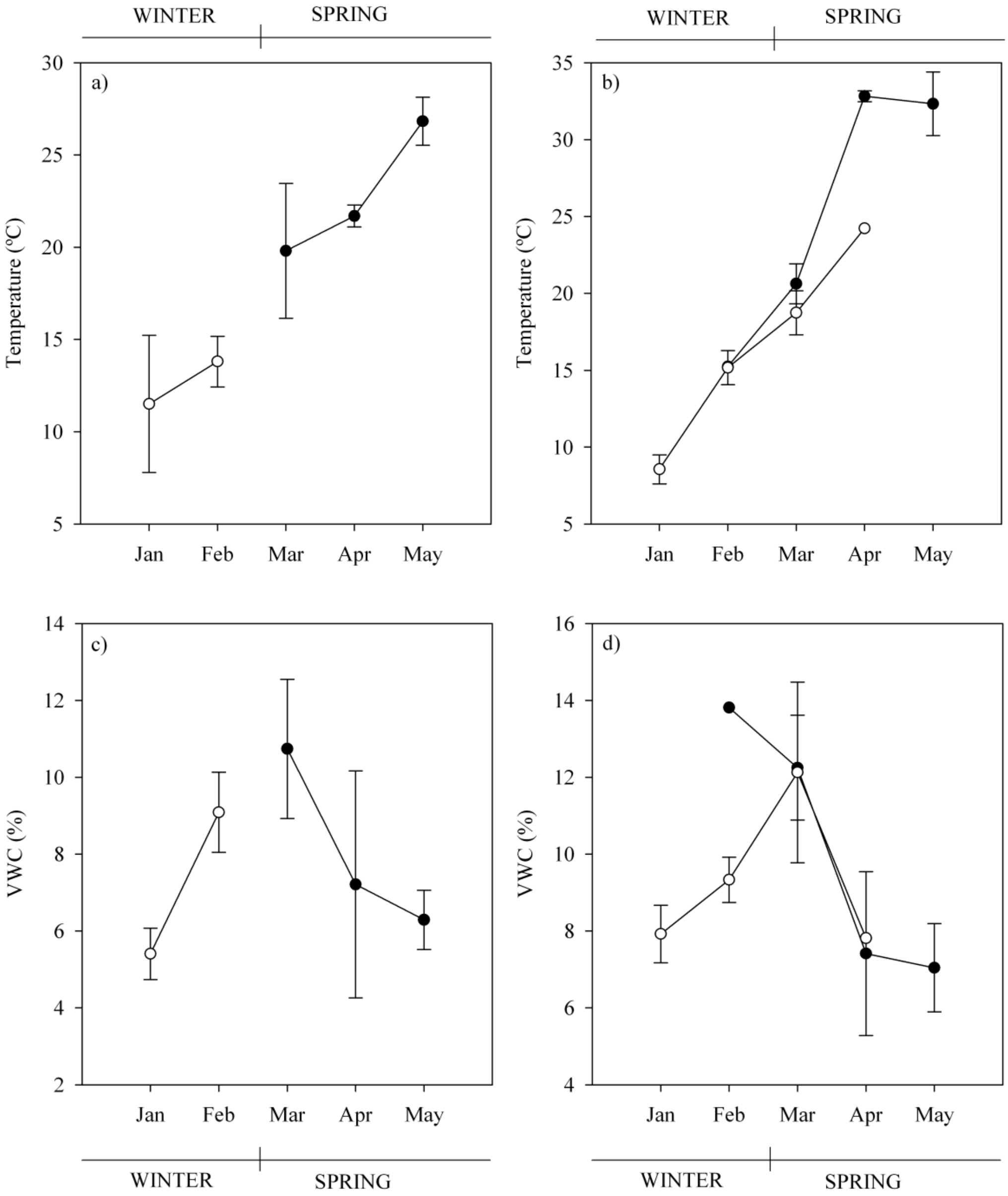


Figure 7. Temperature (ºC) (mean ± SE) (a) in the native and (b) in the invaded-front, and VWC (%) (mean ± SE) (c) in the native and (d) in the invaded-front of *P. pygmaea* active nests (filled dots) and non-active nests (open dots).

Finally, temperatures of active nests between both species and seasons were significantly different **(Table 1)**. In winter the mean soil temperature was the same for both species, while in spring *P. pygmaea* active nests had a higher temperature than *L. humile*. VWC did not differ between active nests of the two species **(Table 1)**. Thus, although VWC was not significantly different, it seems that the Argentine ant tended to nest in colder and therefore more humid locations in spring than *P. pygmaea* **(Figure 8)**.

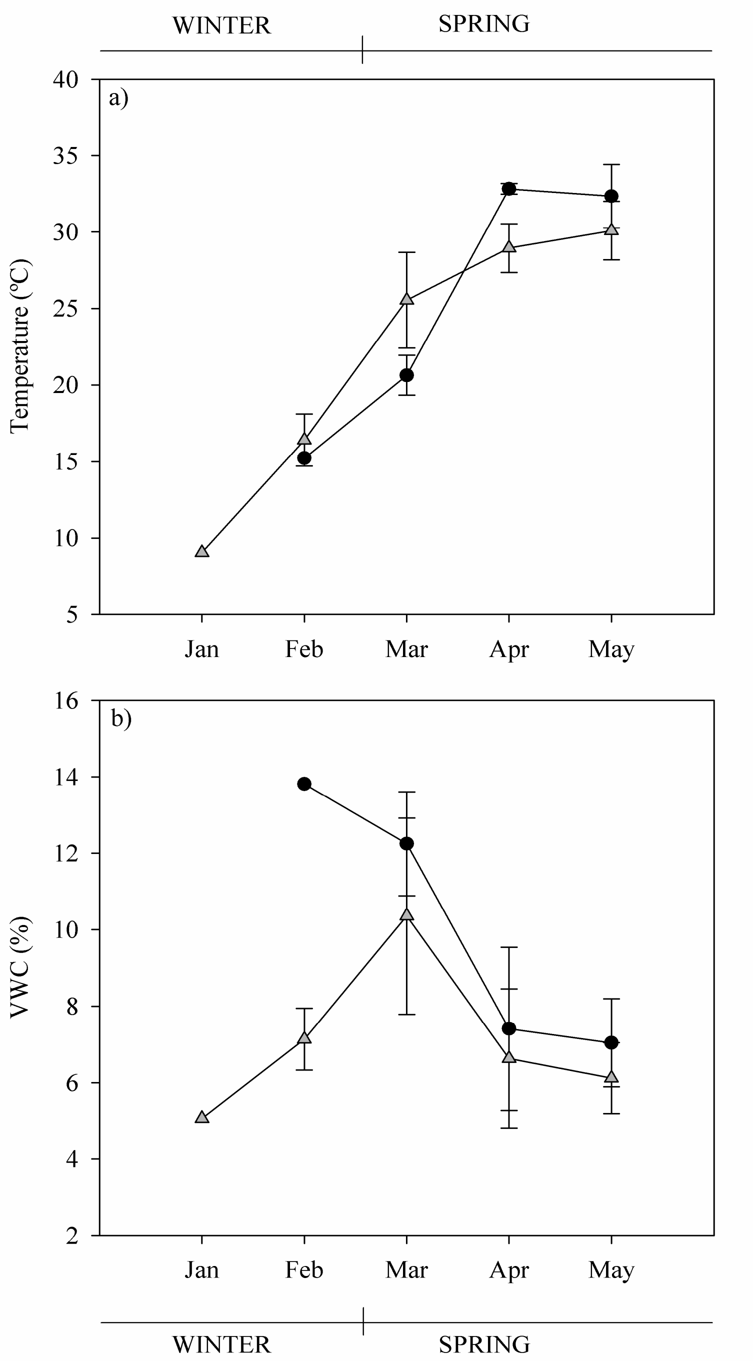


Figure 8. (a) Temperature (ºC) (mean ± SE) and (b) VWC (%) (mean ± SE) of *P. pygmaea* (filled dots) and *L. humile* (grey triangles) active nests in the invaded-front.

4. DISCUSSION

According to Passera et al. (2001), the nests of *P. pygmaea* in this study followed an annual fission-fusion cycle in both sampled zones (native and invaded-front) due to seasonal climate variations. Nests were active from September to October, suffering a great decrease of activity from one month to the next until it was null or almost null in November. In fact, these months constitute the fusion period of the cycle, when *P. pygmaea* fuses the nests from other colony subunits to create large formations known as "winter nests", and whose build-up reduces the level of polydomy for overwintering (Passera et al. 2001) from November to mid-February. During this hibernation period it seems to be no activity inside the nests. This assumption fits with colonies in the native zone, where there was a total lack of activity during this period, and nests actually did not register any visible activity until the end of February. However, in the invaded-front *P. pygmaea* still maintained some activity inside nests in 6.6 to 18.18% of cases. Additionally, in this zone *L. humile* is highly present and seems to take advantage of this *P. pygmaea* hibernation period, expanding and colonizing new areas suitable for winter nesting. This increased *L. humile* density in winter in areas where *P. pygmaea* was present, could have been a consequence of the invader's search for suitable sites for winter nesting. Some of these *L. humile* ants were perhaps just passing through until an optimal site was found to build-up its definitive winter nest. However, in 38.9% of the cases *L. humile* stayed in *P. pygmaea* nests during the whole winter. Most of the *P. pygmaea* nests occupied by the Argentine ant were nests without activity. *L. humile* is known to have a decreased activity or even a lack of hibernation period in areas where it is invasive (Benois 1973, Holway 1998a, Abril et al. 2007) and it profits from the absence of other native ant species activity that could be interacting with it in competition for nesting sites or food resources. Late fall and winter seem to be the periods when the Argentine ant begins to form its winter nests in the invaded area studied. With the warm winter microclimate of the invaded Mediterranean-type areas, and the optimal temperature range for foraging activity during daytime hours (Abril et al. 2007), it may be that these factors are enabling this ant species to colonize new areas and retracting the native species in physiological stop. Thus, one reason why *P. pygmaea* registered some activity inside the nests in the invaded-front during winter may be the presence of *L. humile* during that period. *P. pygmaea* active nests were only occupied by the Argentine ant once, while in 83.3% of cases *L. humile* occupied *P. pygmaea* nests without activity inside them. One explanation could be that maintaining some activity inside the nests helps prevent *L. humile* taking advantage of *P. pygmaea* nests and invading them, since in winter *L. humile* seems to prefer to occupy nests that don’t resist its invasion. This may occur because it is a more vulnerable period of the colony cycle, with a low movement speed at low temperatures that might make workers more susceptible to negative interactions with other ant species (Jumbam et al. 2008).

Until mid-February *P. pygmaea* nests raised and expanded again, searching for new areas to colonize and food resources for the requirements of the colony in spring-summer. In this period of the year, colonies split into numerous nests that remain in close proximity to one another (Passera et al. 2001). Although *P. pygmaea* made the same effort to build new nests in spring in both zones, nest density decreased in the invaded-front zone. Perhaps this was due to the fact that *L. humile* was at the beginning of its reproductive cycle, during which time workers increased their own activity to carry out several tasks, such taking care of the brood or feeding the queens (Abril et al. 2007). Thus, the Argentine ant occupied 24% more *P. pygmaea* active nests in this period than in winter, most likely because the great increase in individuals at this stage of its biological cycle allowed it to be numerically dominant over the native ant species and to improve its invasive power. In addition, *P. pygmaea* tended to renew its nests in the native zone more than in the invaded-front during this period of the year. Most of these nests were not renewed in the invaded-front, possibly because they were occupied by the Argentine ant, which profited from the hibernation period of *P. pygmaea*, and in any case the latter retrieved its old nests occupied by the former.

Although abundance of *P. pygmaea* workers were in the same level in both zones, the pressure of the Argentine ant expanding and colonizing new areas in the invaded-front could influenced the abundance of *P. pygmaea* workers, as individuals decreased by 50% in relation to the native zone while the Argentine ant raised its presence. This result concurs with those found by M.L. Enríquez, S. Abril, M. Diaz & C. Gómez (unpublished), the presence of *P. pygmaea* workers in the invaded-front being lower than in the native one. Although one might surmise that the presence of the Argentine ant is the main reason for the decrease in *P. pygmaea* workers, M.L. Enríquez, S. Abril, M. Diaz & C. Gómez (unpublished) also found that in invaded zones located further from the front of the invasion (i.e. invaded-centre, zones at a mean distance of 450 m, **CHAPTER 3**) , *P. pygmaea* seemed to be less affected than other native ant species that suffered a greatly reduced presence or even a total absence in the zones where the Argentine ant was found. In fact, M.L. Enríquez, S. Abril, M. Diaz & C. Gómez (unpublished) found that *P. pygmaea* increased its presence while other native ants decreased theirs in the invaded-centre, such as *Crematogaster scutellaris* (Olivier 1792), *Pheidole pallidula* (Nylander 1849) or *Camponotus cruentatus* (Latrielle 1802), whose numbers were heavily reduced by the presence of the Argentine ant. One possible explanation for this could be that the presence of more native ant species in the invaded-front than in the invaded-centre means that *P. pygmaea* has more competition pressure for resources, and as it is a submissive species it is disfavoured as much by other species than by the Argentine ant. Moreover, in the invaded-centre *P. pygmaea* increased its presence because its competitors were limited to *L. humile*, which had already retracted other native ant species and left more suitable nesting sites with increased available food resources for *P. pygmaea* to colonize and take advantage of. Additionally, although Abril & Gómez (2009) concluded that the coexistence of the two species cannot be totally explained as a result of a habituation process (as other authors found with other ant species), they observed that the process could also play some role in increasing the *P. pygmaea* presence in a higher number of invaded zones. In fact, they observed that the Argentine ant tends to initiate more aggression when confronted with *P. pygmaea* in the front than when they meet in the centre of the invasion. This could be explained by the fact that by the time they meet in the invaded-centre the Argentine ant has reached a certain degree of habituation, which appears to be an additional factor underlying the coexistence between these two ant species in the invaded zones (Abril & Gómez 2009).

In regard to the physical characteristics of nests, during the entire period sampled *P. pygmaea* in the native zone was observed to nest closer to trees or shrubs providing a close food resource and a high canopy cover above its nests, while in the invaded-front nests were built further from trees, which therefore offered a low canopy cover. These results could be explained by the fact that in the invaded-front *P. pygmaea* has more competitive pressure due to the additional presence of *L. humile*, and thus less optimal sites to nest in. The former locates its nests in areas where there are still suitable nesting sites to colonize that meet its requirements post-hibernation, during which period the Argentine ant will have been gaining the advantage over the rest of the native ants, even occupying some of the *P. pygmaea* non-active nests. In contrast, in the native area, as *P. pygmaea* starts to spread almost at the same time as its native ant competitors, it has more opportunities to choose a better nesting site nearer to trees and shrubs that allow it to forage with the minimum of effort and energy waste. In addition, it seems that these *P. pygmaea* in locations in the invaded-front raised the temperature inside nests. Those found to be active in hottest locations registered a difference of 7ºC of when compared with active nests in the native zone. In contrast, soil moisture stayed within the same range over the sampled period and zones. Moreover, the fact that nest soil water content is related to winter nest temperature ($r^2 = 0.21$, $P = 0.02$) indicates that the latter acts, along with other factors, as a regulator of soil moisture, decreasing high percentages of water content and avoiding high soil water evaporation, therefore preventing desiccation. To prevent over exposure to sun due to large canopy gaps (Retana & Cerdà 2000) in the invaded-front and to avoid critically high temperatures (> 40ºC, Cerdà et al. 1998), *P. pygmaea* tends to nest facing the side with less hours of sunlight during spring. This orientation helps them to achieve the range of optimal temperatures for the colony activity found by Cerdà et al. (1998) of 10 to 36ºC. However, the mean temperature in the invaded-front (29.37 ± 1.65ºC SE) was higher than the maximum activity of temperature foraging (MAT) of 22ºC suggested by Cerdà et al. (1998), while in the native zone (22.77 ± 1.55ºC SE) this temperature concurred with the MAT range for this species. This also could be an additional strategy of *P. pygmaea*, i.e., that it considers it advantageous to avoid intense interference and confrontation with the Argentine ant, as the former changes its foraging activity in the invaded-front at temperatures much closer to its critical thermal tolerance (Cerdà et al. 1998).

On the other hand, we found Argentine ant active nests in winter at locations with fewer hours of sunlight, further from plant structures, and with a higher canopy cover than *P. pygmaea*, though this canopy cover was lower than those we found for winter nests as detailed in **CHAPTER 1**. This exposure to high canopy openness, with the possibility of reaching non-optimal soil temperature ranges in areas where *P. pygmaea* was present in winter, forced *L. humile* to nest mostly facing northerly, and thus avoid reaching critically high temperatures that endangered colony survival (Jumbam et al. 2008). Perhaps these were transition nests until the definitive winter nest was located, and this is the reason why we found differences in canopy cover, distance to the nearest tree, and orientation between *L. humile* winter nests, as discussed in the opening chapter of this work. These active nests in winter were located in places with a mean temperature of 15.49 ±1.74ºC, and this temperature concurred with the mean daily temperature during mid-winter of 16.11 to 17.61ºC mentioned in **CHAPTER 1**. The Argentine ant is more likely to nest in warm sites in winter due to the fact that winter nest soil temperature influences nest location in this period. Additionally, these results fit within the range of temperatures found by Brightwell et al. (2010) for foraging activity during winter in northern California. This suggests that the mid-winter temperatures found in our study area also permitted foraging activity during this period (Abril et al. 2007). In fact, we think this is the main reason why the Argentine ant was active during winter in our study area and it may also explain why it takes advantage of *P. pygmaea* non-active nests. Exactly 21.7% of the Argentine ant nests found in winter were nests pre-built by the other species. This *L. humile* strategy forces the retreat of native ants in invaded natural areas, causing a drastic decrease in the biodiversity of these zones.

We can conclude that *P. pygmaea* is affected by the presence of *L. humile* in the invaded-front. In fact, nest density and worker abundance seems to decrease while the Argentine ant is gaining ground in this area. The added pressure caused by the presence of other native ant species and the higher aggression displayed by *L. humile* against *P. pygmaea* in the edge of the invasion could decimate the presence of the latter in this zone. Such a scenario forces *P. pygmaea* to exploit less optimal nesting sites and resources closer to its critical thermal tolerance (Cerdà et al. 1998). However, in invaded zones further from the edge of the invasion, *P. pygmaea* seems to take advantage of the presence of the Argentine ant, as the latter removes all the other native ant species and consequently *P. pygmaea* experiences a decrease in competitive pressure. Additionally, the habituation possible between these species in invaded zones permits them to coexist in apparent equilibrium (Abril & Gómez, 2009) and may be a side factor contributing to the increase of *P. pygmaea* presence in these areas.

GENERAL DISCUSSION & FUTURE RESEARCH

(picture: Facundo Fatú)

GENERAL DISCUSSION

The research constituted by this thesis is the continuation of numerous studies focused on the improvement of the future management and control of the Argentine ant invasion in natural areas of Mediterranean mixed cork oak and pine forests.

Previous authors observed that Argentine ant invasive colonies vary seasonally (Newell & Barber 1913, Markin 1970, Benois 1973, Heller & Gordon 2006, Abril et al. 2008b) and that the best time to attack the nests in these areas may be in winter (January-March), the period containing a greater density of queens and individuals per nest (Abril et al. 2008b). Taking this into account, in **CHAPTER 1** we determine the physical and environmental characteristics that condition the location of winter nests in native and invaded zones. The correct location of winter nesting sites could be an important issue in reducing the effort required to manage the invasion.

Preferred winter nesting sites for the Argentine ant are mostly influenced by soil moisture and temperature, as well as factors regulating them. A close tree or shrub and southern orientations (in both native and introduced ranges), and canopy cover and rocks (in the introduced range) help to avoid high levels of soil moisture. These characteristics also protect the colony from extreme temperatures, and maintain optimal environmental conditions both inside nests, and for colony activity during winter.

On the other hand, Vogel et al. (2009) suggested that native supercolonies could be compared with the introduced ones in all aspects except their size. The results found in **CHAPTER 1** indicated that Vogel et al. (2009) were right in their assumption. First, we observed that native supercolonies also seem to follow a yearly pattern of fusion-fission of nests, as do introduced colonies. We obtained similar results regarding the spatial range and distribution of native supercolonies, since we found the same length and almost the same distance between them, though with a lower nest density in winter than that noted two years earlier by Vogel et al. (2009). Second, its nesting behaviour in winter is similar to that of invaded areas, as its individual winter nests were located in largely similar sites. Therefore, the success of the Argentine ant as an invasive species relies neither on a shift in social organization nor in a shift in their mode of nesting associated with the introduction to new habitats. Thus, the winter nesting behaviour of the Argentine ant seems to be intrinsic. Moreover, differences in size between native and introduced supercolonies seem to rely on different ecological features (e.g., pressure from other close supercolonies, native competitors, and parasites (Vogel et al. 2009)) that do not allow native supercolonies to grow and expand as much as they do in introduced areas where the ant species is invasive (Suarez et al. 2001).

Additionally, Heller & Gordon (2006) suggested that the Argentine ant has a certain fidelity to its winter nesting sites year after year. In **CHAPTER 1** we also confirmed this assumption. In our study area the Argentine ant returned to the same winter nesting sites year after year. Furthermore, in **CHAPTER 3** we observed that in invaded areas larger nests remain inhabited longer, and in some cases even remaining so during the entire year. Thus, the main reason for these nests being permanent may be their size, as to move such a huge formation would require a greater effort than to move the satellite nests, which are smaller expansions of those winter nests. This lifetime-size relationship suggested that the large permanent nests with a high density of individuals inside them are most probably mother nests (Heller & Gordon 2006, Heller et al. 2008a), which may work as a source of queens for the colonization of new non-invaded areas. With this in mind, focusing management efforts on the elimination of these mother nests could perhaps be an effective way to attack the plague.

It is known that different traits in queen biology (queen density, fat content in queens, the queen/worker thorax volume ratio, and the oviposition rate) are good estimators of the invasive capabilities of a colony, as they are directly related to dispersion speed and successful colony foundation (Hölldobler & Wilson 1977, Keller & Passera 1989, Stille 1996, Bruna et al. 2011). A successful, more efficient elimination of Argentine ant queens in invaded natural environments requires detailed information about their invasive process and its relation to biological traits. **CHAPTER 2** set out to identify these and to discover how the biological strategies adopted by the Argentine ant change in response to different ecological contexts.

The biological differences of the Argentine ant queens that are discussed in **CHAPTER 2** seem to respond to the different ecological contexts found in the three zones studied (native, invaded-front and invaded-centre). The different strategies adopted in each zone would provide the colonies with different skills to compete for available resources and assure the founding of a successful new colony. Therefore, Argentine ant colonies in the native range have a greater number of queens, and they are smaller in size than in both invaded zones. They also have the highest fat content, as well as the largest workers. Colonies from the invaded-front, however, have a greater number of queens, and they are larger than in the invaded-centre, but with the same fat reserves and worker size.

Results found also indicate that the Argentine ant queens in the native range are physiologically and morphologically well adapted to an independent mode of colony founding, while those in the introduced areas seem to occupy a halfway point between independent and dependent, as queens are morphologically qualified to carry out independent colony founding, but not physiologically (Keller & Passera 1989, Stille 1996). The findings seem to suggest that this is may mark a shift in the Argentine ant colony strategy, which has allowed it to become an invasive species in the introduced range. Although it is well-known that the Argentine ant has a dependent mode of colony founding in introduced areas (Hee et al. 2000), there are no studies addressing this issue in the native range, and in this respect further research is needed.

Ecological and biological strategies followed by the Argentine ant (observed in **CHAPTER 1** and **2** have provided new and valuable information about how this species could make adjustments to adapt itself to different ecological contexts, and how we can improve timing and efforts to manage the invasion in areas of natural interest. These changes were mainly focused on biological rather than on ecological strategies (which experienced only minor behavioural variances) to take advantage of all the available resources in the different ecological contexts in which they are nesting.

Once we know where to locate winter nests and which biological strategies the Argentine ant colonies pursue to assure a successful new colony founding, we can initiate methods to control the invasion based on the elimination of queens and workers. Additionally, however, we also need to know when and where it is better to act to manage the invasion in invaded natural areas. In **CHAPTER 3** helps us to answer these questions.

For this purpose, first we determined the population dynamics of the Argentine ant during one year, and second we assessed a control method based on the manual removal of winter nests in order to eliminate queens and their brood during two consecutives winters. We then analyzed the effects of this management over the long term.

As for the population dynamics of the Argentine ant previous to the extirpation, we confirmed the idea of an annual fission-fusion cycle of invasive populations due to seasonal climate (Benois 1973, Heller 2004) with the results of the seasonal variation in nest distribution found in our sampling area. In winter, nests were larger, low in number, and aggregated (fusion period), and workers were limited in number. On the contrary, nests in spring were smaller, more abundant, and randomly distributed (fission period), and workers were high in number (**CHAPTER 3**). Nests were also larger and more abundant at locations and plots where the competitive pressure to which the Argentine ant was subject to was low. In contrast, nests were smaller and less abundant at the front of the invasion, where the native ant community was better structured and where it exercised a high competitive pressure on the invasive ant (**CHAPTER 3**).

On the other hand, the manual removal of winter nests produced changes mainly in nest size. Nests at treated plots were small and similar along the invasion front, while nests at non-treated plots followed the seasonal cycle of contraction-dispersion and were smaller as we moved closer to the edge of the invasion front. We also observed that larger nests persisted throughout the cold season or even the whole year (Heller & Gordon 2006), indicating that they act as mother nests (Heller & Gordon 2006, Heller et al. 2008a). Nevertheless, the diminished number of these longer lasting nests in treated plots compared with control plots suggests that the extirpation conducted could be dividing mature old nests, making them less persistent. Two-year management would appear to be effective only at the edge of the invasion front, where the Argentine ant population is less structured, weaker, and more vulnerable to disturbance. Extirpation at the edge of the invasion front promotes changes in spatial distribution and the number and size of nests, as well as the time nests remain active over the sampling period, and in the abundance of workers. Manual removal of nests in winter probably reduces the number of spreading gynes and it changes worker priorities, slowing the dispersal capacity of the colony and delaying it.

The first two winters the number of ants decreased after the extirpation at treated plots, but nests and workers recovered again in the third winter, indicating that to keep the number of nests and consequently the expansion of the Argentine ant at a low rate, a yearly extirpation is required.

The effects on Argentine ant population dynamics in nests where extirpations were conducted suggested that it would be best to focus the management of the invasion in winter at the edge of the front, and to do it periodically. However, we think that due to the observed high levels of invasion in our study area, the manual removal of nests should be accompanied by other alternatives methods in order to improve its effectiveness. For example, the use of artificial nests as traps (M.L. Enríquez, S. Abril, M. Diaz & C. Gómez, unpubl.), as well as the integration of other biological control methods such as foraging disruption (Nishisue et al. 2010) or diet dissociation (Silverman & Liang 2001, Guerrieri et al. 2009) in spring could be a way to enhance winter nest extirpation, keep the front stationary and prevent the ant from invading new sites.

As a consequence of the nest exchange observed between the Argentine ant and *P. pygmaea*, (detailed in **CHAPTER 1**), and as the coexistence of these two species in invaded natural areas is well-known (Oliveras et al. 2005, Abril & Gómez 2009), other questions arose for us. **CHAPTER 4** helps us to better understand the interaction between these two ant species, determine which factors promote this nesting site exchange, and discern whether the submissive ant *P. pygmaea* is benefited or damaged by the presence of the Argentine ant in invaded natural areas.

Results found suggest that the different ecological strategies employed by *P. pygmaea* in the invaded-front in relation to nesting sites seem to permit coexistence in areas where the Argentine ant is present. First, there is the plasticity in its nesting behaviour with regard to physical characteristics of the nesting site, and its having to make do with the less optimal available nesting sites left by the Argentine ant. Second, there is the plasticity in its colony activity, which changes at temperatures much closer to its critical thermal tolerance (Cerdà et al. 1998) in order to avoid intense interference confrontation with the invasive species.

However, in invaded areas further from the edge of the invasion front, *P. pygmaea* seems to be relatively unaffected, increasing its presence while other native ant species decrease theirs (M.L. Enríquez, S. Abril, M. Diaz & C. Gómez, unpubl.). The more structured ant community offers greater competitive pressure for resources, and thus *P. pygmaea* seems to be disfavoured by the presence of several native ants due to its submissive nature. Nevertheless, in an unstructured ant community this tiny ant increases its presence due to increased available food resources left by the native ant species eliminated by the Argentine ant. Additionally, a certain degree of habituation between *P. pygmaea* and *L. humile* in invaded zones also could play some role in increasing the presence of the former (Abril & Gómez 2009) in this invaded areas.

FUTURE RESEARCH

The information contained in this thesis relating to improving the timing and efforts made to manage the Argentine ant invasion in invaded natural areas generates new questions which could be answered with future research. Given the high invasion levels found in our study area, we think that the manual removal of nests should be accompanied by other, alternative methods in order to improve its effectiveness. It would be interesting to determine with more precision the effect of integrated biological control methods on queen populations in the edge of the invasion front. For this purpose, we could evaluate queen population growth rate and abundance after assessing an integrated control based on a yearly extirpation of nests, complemented with targeted artificial nests traps (M.L. Enríquez, S. Abril, M. Diaz & C. Gómez, unpubl.) in winter together with diet dissociation (Silverman & Liang 2001, Guerrieri et al. 2009) steps, and foraging disruption (Nishisue et al. 2010) in spring. This integrated control would help us attack the Argentine ant population in winter more effectively and in less time, as well as destructure the population and also decrease the chances of an efficient intake of available food resources in the front of the invasion in spring. Taken together these actions could contribute to diminishing the invasive power of this species at the edge of the invasion front more quickly and successfully, and stop its expansion into new areas.

Second, it would also be interesting to determine the modes of colony founding in the native range of this ant species. Until now there have been no studies addressing this essential issue, knowledge of which could help us to better understand the invasion process in natural environments and truly discover whether the energy investment of the colony has changed in the process of becoming an invasive species. For this purpose, different studies could be carried out based on whether queens leave the nest alone or accompanied by workers. If the Argentine ant ultimately has a dependent mode of colony founding in its native range, how many workers leave the nest initially and how many arrive at the new nesting site? Is this number influenced by intra- and interspecific competition, increasing the mortality rate of workers following the queens? And if this is the case, how many workers are needed to successfully start a new colony in the native range?

Third, as mother nests seem to be a source of queens for the colonization of new non-invaded areas in spring, and they are located in permanent sites year after year, future research on their dynamics could be interesting. Moreover, focusing management efforts on the elimination of these mature old nests may be another effective way to attack the plague. For this purpose, we could specifically determine just how and where they are built, and why they are permanent. Additionally, we could also assess the differences in number, density, and productivity of queens in comparison to non-permanent nests.

Fourth, increased knowledge about how the exchange of nesting sites between the Argentine ant and *P. pygmaea* is promoted could be valuable to help better understand the migration processes and nesting requirements of the Argentine ant. Moreover, this information could be taken into account when conducting future control methods based on targeted artificial nests traps.

Fifth, considering that *P. pygmaea*, also have be seen sharing nest sites (i.e. sheltering rocks) with other native ant species, as *Camponotus cruentatus* (Latreille 1802) or *Aphaenogaster subterranea* (Latreille 1798), it would be interesting assessed why and how this tiny ant could avoid confrontation against some of these other ant species and manages to remain on these sites.

Finally, another possibly study would address worker size, as we found that introduced colonies produce smaller workers than native ones. Taking this into account, we could determine why worker size differs between both ranges, and if it is related to an increased interference competition capability in their native range and/or to an increased invasiveness potential in the areas where this species has been introduced.

CONCLUSIONS

(picture: Alex Wild)

CONCLUSIONS

1. Winter nesting sites are mostly influenced by soil moisture and temperature, as well as factors which can regulate them, such as a relative distance to the nearest tree and orientation (in both native and introduced ranges), and canopy cover and rocks (in the introduced range). All these characteristics help to ensure best conditions inside the nest for colony activity and survival in winter, maintaining temperature and therefore soil moisture in optimal ranges.

2. Winter nesting sites are similar in both introduced and native ranges and differences observed in some of the factors studied here are due to the contrasting ecological contexts of the native and invaded ranges. Thus, the success of the Argentine ant as an invasive species does not rely on a shift in social organization nor in a shift in the mode of nesting associated with the introduction to new habitats. Thus, winter nesting behaviour of the Argentine ant in the invaded areas seems to be intrinsic and it needs only to make minor behavioural adjustments to allow it to take advantage of all the available resources in the different ecological contexts in which they are nesting.

3. The Argentine ant returns to the same nesting site year after year, and may even remain in the same place for the whole cycle. These permanent nests are the largest ones and it seems that they act as mother nests, which provide a major source of queens for the colonization of new non-invaded areas.

4. Biological differences also seem to respond to the different ecological contexts of the studied zones. Native colonies have a greater number of

queens and they are smaller in size than in the invaded ones. They also have the highest fat content and the largest workers. Colonies from the invaded–front, however, have a greater number of queens, and they are larger than in the invaded-centre, but with the same fat reserves and worker size.

5. The Argentine ant queens in the native range could be physiologically and morphologically well adapted to an independent mode of colony founding, while those in the introduced areas seem to occupy a halfway point between independent and dependent, as queens are morphologically qualified to carry out independent colony founding, but not physiologically. The findings seem to suggest that this may mark a shift in the Argentine ant colony strategy, which has allowed it to become an invasive species in the introduced range.

6. Spatial dynamic of the nests of the Argentine ant are influenced by seasonal variations in local climate and interspecific competition exercised by native ants. Nest abundance and nest size decrease from winter to spring and as we get closer to the edge of the invasion front.

7. Two-year management by means of manual removal of nests in winter would appear to be effective only at the edge of the invasion front where the Argentine ant population is less structured, weaker, and more vulnerable to disturbance. Extirpation in these areas promotes changes in spatial distribution, number and size of nests, as well as in the time that nests remain active over the sampling period, and in the abundance of workers. Manual removal of nests in winter probably reduces the number of spreading gynes and changes worker priorities, slowing the dispersal capacity of the colony and delaying it.

8. During the two first winters the number of ants at control plots augmented typically from winter to spring, while treated plots experienced a decrease in workers after extirpation. However, nests and workers at treated plots recovered again in the third winter, indicating that to achieve significant results and to keep the number of nests and consequently the expansion of the Argentine ant at a low level rate, yearly extirpation is required.

9. *P. pygmaea* is affected by the presence of *L. humile* in the invaded-front. Nest density and worker abundance seems to decrease while the Argentine ant is gaining ground in this zone. The high pressure deriving from the presence of other native ants and the greater aggression of *L. humile* against *P. pygmaea* in the invaded-front can decimate the presence of the latter there, and force it to exploit less optimal nesting sites and food resources closer to its critical thermal tolerance.

10. *P. pygmaea* in invaded zones further from the edge of the invasion (i.e. invaded-centre) seems to take advantage of the presence of the Argentine ant, as the latter retracts almost all the other native ant species and consequently there is a decrease of competitive pressure on *P. pygmaea*. Additionally, the habituation possible between these species in the invaded-centre permits them to coexist in apparent equilibrium and it may be a side factor contributing to the increase of *P. pygmaea* presence in these zones.

REFERENCES

REFERENCES

ABRIL S & GÓMEZ C (2009) Ascertaining key factors behind the coexistence of the native ant species *Plagiolepis pygmaea* with the invasive argentine ant *Linepithema humile* (Hymenoptera: Formicidae) Sociobiology 53:559.

ABRIL S, OLIVERAS J, & GÓMEZ C (2007) Foraging activity and dietary spectrum of the Argentine ant (Hymenoptera: Formicidae) in invaded natural areas of the northeast Iberian Peninsula. Environmental Entomology 36:1166.

ABRIL S, OLIVERAS J & GÓMEZ C (2008a) Effect of temperature on the oviposition rate of Argentine ant queens (*Linepithema humile* Mayr) under monogynous and polygynous experimental conditions. Journal of Insect Physiology 54:265.

ABRIL S, OLIVERAS J & GÓMEZ C (2008b) Effect of seasonal dynamics on queen densities of the Argentine ant (*Linepithema humile*) (Hymenoptera: Formicidae) in an invaded natural area of the NE Iberian Peninsula. Sociobiology 51:645-655.

ABRIL S, OLIVERAS J & GÓMEZ C (2010) Effect of temperature on the Argentine ant's development and survival–an experimental study. Journal of Insect Science 10:97, DOI: insectscience.org/10.97.

BENOIS A (1973) Influence of ecological factors on the annual cycle and seasonal activity of the Argentine ant, *Iridomyrmex humilis* Mayr (Hymenoptera, Formicidae) from the French Riviera. Insectes Sociaux 20:267.

BERNARD F (1968) Les fourmis (Hymenoptera: Formicidae) d'Europe occidentale et septentrionale. Faune de I'Europe et du Bassin méditerranéen 3:411.

BLANCAFORT X & GÓMEZ C (2005) Consequences of the Argentine ant, *Linepithema humile* (Mayr), invasion on pollination of euphorbia *Characias (L.)* (Euphorbiaceae). Acta Oecologica 28:49-55.

BOND W & SLINGSBY P (1984) Collapse of ant-plant mutualism: The Argentine ant (*Iridomyrmex humilis*) and Myrmecochorous proteaceae. Ecology 65:1031-1037.

BRIGHTWELL RJ, LABADIE PE & SILVERMAN J (2010) Northward expansion of the invasive *Linepithema humile* (Hymenoptera: Formicidae) in the Eastern United States is constrained by winter soil temperatures. Environmental Entomology 39:1659-1665.

BRUNA EM, IZZO TJ, INOUYE BD, URIARTE M & VASCONCELOS HL (2011) Asymmetric dispersal and colonization success of Amazonian plant-ants queens. PLoS ONE 6(8): e22937. doi:10.1371/journal.pone.0022937.

CABRERA S (2009) Diversidad y patrones de organización en ensambles de hormigas terrestres (omnívoras) de la Reserva Natural Otamendi. Tesis de Licenciatura, Fac. Cs Exactas y Naturales, Universidad de Buenos Aires.

CARDÉ RT (1990) Principles of mating disruption. R. L. Ridgway, R. M. Silverstein, and M. N. Inscoe (Eds.), Behavior-Modifying Chemicals for Insect Management: Application of Pheromones and Other Attractants. Marcel Dekker, New York. 47-71.

CARPINTERO S & REYES-LÓPEZ J (2008) The role of competitive dominance in the invasive ability of the Argentine ant (*Linepithema humile*). Biological Invasions 10:25-35.

CASELLAS D (2004) Tasa de expansión de la hormiga Argentina, *Linepithema humile* (Mayr 1868), (Hymenoptera: Dolichoderine) en un área mediterránea. Boletín de la Asociación Española de Entomología 28:207-216.

CERDÀ X (1997) Thermal disruption of transitive hierarchies in Mediterranean ant communities. Journal of Animal Ecology 66:363-374.

CERDÀ X, RETANA J & CROS S (1998) Critical thermal limits in Mediterranean ant species: trade-off between mortality risk and foraging performance. Functional Ecology 12:45-55.

CERDÀ X, DAHBI A & RETANA J (2002) Spatial patterns, temporal variability, and the role of multi-nest colonies in a monogynous Spanish desert ant. Ecological Entomology 27:7-15.

CLAVERO M & BROTONS J (2010) Functional homogenization of bird communities along habitat gradients: accounting for niche multidimensionality. Global Ecology and Biogeography 19:684-696.

CLARK PJ & EVANS FC (1954) Distance to nearest neighbour as a measure of spatial relationships in populations. Ecology 35:445-453.

COLE FR, MEDEIROS AC, LOOPE LL & ZUEHLKE WW (1992) Effects of the Argentine ant on arthropod fauna of Hawaiian high-elevation shrubland. Ecology 734:1313–1322.

CHICHIZOLA SE (1993) Las comunidades vegetales de la Reserva Natural Estricta Otamendi y sus relaciones con el ambiente. Parodiana 8:227-263.

DAANE KM, SIME KR, FALLON J & COOPER ML (2007) Impacts of Argentine ants on mealybugs and their natural enemies in California's coastal vineyards. Ecological Entomology 32:583-596.

ESPADALER X & COLLINGWOOD CA (2000) Transferred ants in the Iberian Peninsula (Hymenoptera: Formicidae). Nouvelle Revue d'Entomologie 3:257-263.

ESPADALER X & GÓMEZ C (2003) The Argentine ant, *Linepithema humile*, in the Iberian Peninsula. Sociobiology 42:187-192.

ESPADALER X & REY S (2001) Biological constraints and colony founding in the polygynous invasive ant *Lasius neglectus* (Hymenoptera: Formicidae). Insectes Sociaux 48:159-164.

ESTANY-TIGERSTRÖM D, BAS JM & PONS P (2010) Does Argentine ant invasion affect prey availability for foliage-gleaning birds? Biological Invasions 12:827-839.

FERNÁNDEZ-ESCUDERO I & TINAUT A (1999) Factors determining nest distribution in the high-mountain ant *Proformica longiseta* (Hymenoptera: Formicidae). Ethology Ecology & Evolution 11:325-338

FERNÁNDEZ-ESCUDERO I, TINAUT A & RUANO F (1993) Rock selection for nesting in *Proformica longiseta* Collingwood, 1978 (Hymenoptera: Formicidae) in a Mediterranean high mountain. International Journal of Biometeorology 37:83-88.

FONT DE MORA R (1923) Sobre la presencia de la hormiga Argentina (*Iridomyrmex humilis*) en Valencia. Boletín de la Real Sociedad Española de Historia Natural 23:77-78.

GARCÍA-MERCET R (1921) La hormiga argentina de Oporto. Asociación Española Programa de Ciencias, Congreso de Oporto 6:99-104.

GIRAUD T, PEDERSEN JS & KELLER L (2002) Evolution of supercolonies: The Argentine ants of southern Europe. Proceedings of the National Academy of Science 99:6075-6079.

GÓMEZ K & ESPADALER X (2006) Exotic ants (Hymenoptera: Formicidae) in the Balearic Islands. Myrmecologische Nachricten 8:225–233.

GÓMEZ C & OLIVERAS J (2003) Can the Argentine ant (*Linepithema humile* Mayr) replace native ants in myrmecochory? Acta Oecologica 24:47-53.

GÓMEZ C, PONS P & BAS JM (2003) Effects of the Argentine ant *Linepithema humile* on seed dispersal and seedling emergence of *Rhamnus alaternus*. Ecography 26:532-538.

GOVETO L, SAIBENE C, MOREYRA P, VILLARREAL N, NALE ME, ROMITTI M, MÉNDEZ M & CAMPOS M (2008) Ficha informativa de los humedales de Ramsar (FIR). Reserva Natural Otamendi, Buenos Aires, Argentina.

GRANGIER J, LEBRETON J, DEJEAN A & ORIVEL J (2007) Coexistence between *Cyphomyrmex* ants and dominant populations of *Wasmannia auropunctata*. Behavioural Processes 74:93-96.

GUERRIERI FJ, NEHRING V, JØRGENSEN CG, NIELSEN J, GALIZIA CG & D'ETTORRE P (2009) Ants recognize foes and not friends. Proceedings of the Royal Society B: Biological Sciences 276:2461-2468.

HARRIS RJ (2002) Potential impact of the Argentine ant (*Linepithema humile*) in New Zealand and options for its control. Science for Conservation 196:36.

HARTLEY S & LESTER PJ (2003) Temperature-dependent development of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae): a degree-day model with implications for range limits in New Zealand. New Zealand Entomologist 26:91-100.

HEE JJ, HOLWAY DA, SUAREZ AV & CASE TJ (2000) Role of propagule size in the success of incipient colonies of the invasive Argentine ant. Conservation Biology 14:559-563.

HELLER NE (2004) Colony structure in introduced and native populations of the invasive Argentine ant, *Linepithema humile*. Insectes Sociaux 51:378-386.

HELLER NE & GORDON DM (2006) Seasonal spatial dynamics and causes of nest movement in colonies of the invasive Argentine ant (*Linepithema humile*). Ecological Entomology 31:499-510.

HELLER NE, SANDERS NJ & GORDON DM (2006) Linking temporal and spatial scales in the study of an Argentine ant invasion. Biological Invasions 8:501-507.

HELLER NE, INGRAM KK & GORDON DM (2008a) Nest connectivity and colony structure in unicolonial Argentine ants. Insectes Sociaux 55:397-403.

HELLER NE, SANDERS NJ, WADE SHORS J & GORDON DM (2008b) Rainfall facilitates the spread, and time alters the impact, of the invasive Argentine ant. Oecologia 155:385.

HOLWAY DA (1998a) Factors governing rate of invasion. A natural experiment using Argentine ants. Oecologia 115:206-212.

HOLWAY DA (1998b) Effect of Argentine ant invasions on ground-dwelling arthropods in northern California riparian woodlands. Oecologia 116:252-258.

HOLWAY DA, SUAREZ AV & CASE TJ (1998) Loss of intraspecific aggression in the success of a widespread invasive social insect. Science 282:949-952.

HOLWAY DA, SUAREZ A & CASE TJ (2002a) Role of abiotic factors in governing susceptibility to invasion: a test with Argentine ants. Ecology 83:1610-1619.

HOLWAY DA, LACH L, SUAREZ AV, TSUTSUI ND & CASE TJ (2002b) The causes and consequences of ant invasions. Annual Review of Ecology & Systematics 33:181-233.

HÖLLDOBLER B & WILSON EO (1977) The number of queens: an important trait in ant evolution. Naturwissenschaften 64:8-15

HÖLLDOBLER B & WILSON EO (1990) The ants. The Belknap Press of Harvard University Press, Cambridge, MA.

HOOPER-BUI L & RUST MK (2000) Oral toxicity of abamectin, boric acid, fipronil, and hydramethylnon to laboratory colonies of Argentine ants (Hymenoptera: Formicidae). Journal of Economic Entomology 93:858-864.

HUMAN KG & GORDON DM (1996) Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile,* and native ant species. Oecologia 105:405-412.

HUMAN KG & GORDON DM (1997) Effects of Argentine ants on invertebrate biodiversity in northern California. Conservation Biology 11:1242-1248.

HUMAN KG & GORDON DM (1999). Behavioral interactions of the invasive Argentine ant with native ant species. Insectes Sociaux 46:159-163.

INGRAM KK (2002a) Plasticity in queen number and social structure in the invasive Argentine ant (*Linepithema humile*). Evolution 56:2008-2016.

INGRAM KK (2002b) Flexibility in nest density and social structure in invasive populations of the Argentine ant, *Linepithema humile*. Oecologia 133:492.

JUMBAM KR, JACKSON S, TERBLANCHE JS, MCGEOCH MA & CHOWN SL (2008) Acclimation effects on critical and lethal thermal limits of workers of the Argentine ant, *Linepithema humile*. Journal of Insect Physiology 54:1008-1014.

KELLER L (1995) Social life: The paradox of multiple-queen colonies. Trends in ecology evolution 10:355-360.

KELLER L & PASSERA L (1988) Energy investment in gynes of the Argentine ant *Iridomyrmex humilis* (Mayr) in relation to the mode of colony founding in ants (Hymenoptera: Formicidae). International Journal of Invertebrate Reproduction and Development 13:31-38.

KELLER L & PASSERA L (1989) Size and fat content of gynes in relation to the mode of colony founding in ants (Hymenoptera; Formicidae). Oecologia 80:236-240.

KELLER L, PASSERA L & SUZZONI JP (1989a) Queen execution in the Argentine ant, *Iridomyrmex humilis*. Physiological Entomology 14:157-163.

KELLER L, CHERIX D & ULLOA-CHACÓN P (1989b) Description of a new artificial diet for rearing ant colonies as *Iridomyrmex humilis, Monomorium pharaonis* and *Wasmannia auropunctata* (Hymenoptera: Formicidae). Insectes Sociaux 36:348-352.

KLOTZ JH, GREENBERG L, AMRHEIN C, RUST MK (2000) Toxicity and repellency of borate-sucrose water baits to Argentine ants (Hymenoptera: Formicidae). Journal of Economic Entomology 93:1256-1258.

KOLAR CS & LODGE DM (2001) Progress in invasion biology: predicting invaders. Trends in Ecology & Evolution 16:199-204.

KRUSHELNYCKY PD & REIMER NJ (1998a) Efficacy of maxforce bait for control of the Argentine ant (Hymenoptera: Formicidae) in Haleakala national park, Maui, Hawaii. Environmental Ecology 27:1473-1481.

KRUSHELNYCKY PD & REIMER NJ (1998b) Bait preference by the Argentine ant (Hymenoptera: Formicidae) in Haleakala national park, Hawaii. Environmental Ecology 27:1483-1487.

LIANG D & SILVERMAN J (2000) "You are what you eat": Diet modifies cuticular hydrocarbons and nestmate recognition in the Argentine ant, *Linepithema humile*. Naturwissenschaften 87:412-416.

LOWE S, BROWNE M, BOUDJELAS S & DE POORTER M (2000) 100 of the World’s Worst Invasive Alien Species a Selection from the Global Invasive Species Database. IUCN, New Zealand. − http://www.issg.org/database, available online.

MACK RN, SIMBERLOFF D, LONSDALE WM, EVANS H, CLOUT M & BAZZAZ FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 10:689-710.

MARKIN GP (1970) The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis*, (Hymenoptera: Formicidae), in southern California. Annals of the Entomological Society of America 63:1238-1242.

MARTÍNEZ MD, ARNALDOS MI, ROMERA E, GARCÍA MD (2002) Los Formicidae (Hymenoptera) de una comunidad sarcosaprófaga en un ecosistema mediterráneo. Anales de Biología 24:33-44.

MARTÍNEZ-DUEÑAS WA (2004) INRA–Relative iNtegrated anthropization index: A conceptual technical proposal and its application. Intropica: Revista Del Instituto De Investigaciones Tropicales 5:37-46.

MARTINS MN (1907) Une fourmi terrible envahissant l’Europe (*Iridomyrmex humilis* Mayr). Brotéria 6:101-106.

MCCAFFREY J & GALEN C (2011) Between a Rock and a Hard Place: Impact of Nest Selection Behavior on the Altitudinal Range of an Alpine Ant, *Formica neorufibarbis*. Environmental Entomology 40:534-540.

MCGLYNN T (1999). The world transfer of ants: Geographical distribution and ecological invasions. Journal of Biogeography 26:535-548.

MCKINNEY ML & LOCKWOOD JL (1999) Biotic homogenization: a few winners replacing many losers in the next mass extinction. Trends in Ecology & Evolution 14:450-453.

MENKE SB & HOLWAY DA (2006) Abiotic factors control invasion by Argentine ants at the community scale. Journal of Animal Ecology 75:368-376.

MENKE SB, FISHER RN, JETZ W & HOLWAY DA (2007) Biotic and abiotic controls of argentine ant invasion success at local and landscape scales. Ecology 88:3164-3173.

MERCIER B, PASSERA L & SUZZONI JP (1985) Study of the polygyny of the ant *Plagiolepis pygmaea* Latr. (Hymenoptera: Formicidae): I. The fecundity of the queens reared in monogynous experimental condition. Insectes Sociaux 32:335-348.

MYERS JH, SIMBERLOFF D, KURIS AM & CAREY JR (2000) Eradication revisited: dealing with exotic species. Trends in Recent Ecology & Evolution 15:316–320.

NEWELL W & BARBER TC (1913) The Argentine ant, USDA. Bureau of Entomology Bulletin 122:1-98.

NISHISUE K, SUNAMURA E, TANAKA Y, SAKAMOTO H, SUZUKI S, FUKUMOTO T, TERAYAMA M & TATSUKI S (2010) Long-term field trial to control the invasive Argentine ant (Hymenoptera: Formicidae) with synthetic trail pheromone. Journal of Economic Entomology 103:1784-1789.

NOBEL PS, MILLER PM & GRAHAM EA (1992) Influence of Rocks on Soil-Temperature, Soil-Water Potential, and Rooting Patterns for Desert Succulents. Oecologia 92:90-96.

OLIVERAS J, BAS JM, CASELLAS D & GÓMEZ C (2005) Numerical dominance of the Argentine ant vs native ants and consequences on soil resource searching in

Mediterranean Cork-oak forest (Hymenoptera: Formicidae). Sociobiology 45:643-658.

ORR MR, SEIKE SH, BENSON WW & DAHLSTEN DL (2001) Host specificity of *Pseudacteon* (Diptera: Phoridae) parasitoids that attack *Linepithema* (Hymenoptera: Formicidae) in South America. Environmental Entomology 30:742-747.

PASSERA L (1963) Le cycle évolutif de la fourmi *Plagiolepis pygmæa*. Insectes Sociaux 10:59-69.

PASSERA L (1969) Interactions et fécondité des reines de *Plagiolepis pygmæa* Latr. et de ses parasites sociaux *P. Grassei* le masne et passera et *P. xene* St. (Hymenoptera: Formicidæ). Insectes Sociaux 16:179-193.

PASSERA L (1994) Characteristics of tramp species. In: Williams DF (ed) exotic ants: Biology, impact, and control of introduced species. 23-43.

PASSERA L, GILBERT M & ARON S (2001) Social parasitism in ants: Effects of the inquiline parasite *Plagiolepis xene* St. on queen distribution and worker production of its host *Plagiolepis pygmaea* Latr. Insectes Sociaux 48:74-79.

PEAKIN GJ (1972) Aspects of productivity in *Tetramorium caespitum* L. Ekologia Polska 20:55-63.

PEDERSEN JS, KRIEGER MJB, VOGEL V, GIRAUD T & KELLER L (2006) Native supercolonies of unrelated individuals in the invasive Argentine ant. Evolution 60:782-791.

PIMENTEL D, LACH L, ZUNIGA R & MORRISON D (2000) Environmental and economic costs of nonindigenous species in the United States. BioScience 50:53-65.

PONS X (2011) MiraMon 7.0. Geographical information system and teledetection software. CREAF, Bellaterra, Spain.

R DEVELOPMENT CORE TEAM. (2010) R, A language and environment for statistical computing. R Foundation for Statistical Computing. http://www.R-project.org.

RETANA J & CERDÀ X (2000) Patterns of diversity and composition of Mediterranean ground ant communities tracking spatial and temporal variability in the thermal environment. Oecologia 123:436-444.

REUTER M, PEDERSEN JS & KELLER L (2005) Loss of *Wolbachia* infection during colonisation in the invasive Argentine ant *Linepithema humile*. Heredity 94:364–369.

ROBINSON WA (2008) Selection and sharing of sheltered nest sites by ants (Hymenoptera: Formicidae) in grasslands of the Australian Capital Territory. Australian Journal of Entomology 47:174-183.

RODRÍGUEZ-CABAL M, STUBLE KL, NUÑEZ MA & SANDERS NJ (2009) Quantitative analysis of the effects of the exotic Argentine ant on seed dispersal mutualisms. Biology Letters 5:499-502.

ROURA-PASCUAL N, BAS JM & HUI C (2010). The spread of the Argentine ant: environmental determinants and impacts on native ant communities. Biological Invasions 12:2399-2412.

ROURA-PASCUAL N, SUAREZ AV, GÓMEZ C, PONS P, TOUYAMA Y, WILD AL & PETERSON AT (2004) Geographical potential of Argentine ants (*Linepithema humile* Mayr) in the face of global climate change. Proceedings of the Royal Society B: Biological Sciences 271:2527-2534.

ROURA-PASCUAL N, SUAREZ AV, MCNYSET K, GÓMEZ C, PONS P, TOUYAMA Y, WILD AL, GASCÓN F & PETERSON AT (2006) Niche differentiation and fine-scale projections for Argentine ants based on remotely sensed data. Ecological Applications 16:1832-1841.

ROURA-PASCUAL N, HUI C, IKEDA T, LEDAY G, RICHARDSON DM, CARPINTERO S, ESPADALER X, GÓMEZ C, GUÉNARD B, HARTLEY S, KRUSHELNYCKY P, LESTER PJ, MCGEOCH MA, MENKE S, PEDERSEN J, PITT J, REYES J, SANDERS NJ, SUAREZ AV, TOUYAMA Y, WARD DF, WARD P & WORNER SP (2011) The relative roles of climatic suitability and anthropogenic influence in determining the pattern of spread in a global invader. Proceedings of the National Academy of Sciences of the United States of America 108:220-225.

SANDERS NJ, GOTELLI NJ, HELLER NE & GORDON DM (2003) Community disassembly by an invasive ant species. Proceedings of the National Academy of Sciences of the United States of America 100:2474–2477.

SCHILMAN PE, LIGHTON JRB & HOLWAY DA (2007) Water balance in the Argentine ant (*Linepithema humile*) compared with five common native ant species from southern California. Physiological Entomology 32:1-7.

SERRANO JM, ACOSTA FJ & ÁLVAREZ M. (1987) Estructura de la comunidades de hormigas en eriales mediterráneos según criterios funcionales. Graellsia 43:211-223.

SILVERMAN J & LIANG D (2001) Colony disassociation following diet partitioning in a unicolonial ant. Naturwissenschaften 88:73-77.

SILVERMAN J & BRIGHTWELL RJ (2008) The Argentine ant: Challenges in managing an invasive unicolonial pest. Annual Reviews Entomology 53:231–52.

STILLE M (1996) Queen/worker thorax volume ratios and nest-founding strategies in ants. Oecologia 105:87-93.

SUAREZ AV, BOLGER DT & CASE TJ (1998) Effects of fragmentation and invasion on native ant communities in coastal southern California. Ecology 79:2041-2056.

SUAREZ AV, RICHMOND JQ & CASE TJ (2000) Prey selection in horned lizards following the invasion of Argentine ants in southern California. Ecological Applications 10:711-725.

SUAREZ AV, HOLWAY DA & CASE TJ (2001) Patterns of spread in biological invasions dominated by long-distance jump dispersal: Insights from Argentine ants. Proceedings of the National Academy of Sciences of the United States of America 98:1095-1100.

SUAREZ AV, TSUTSUI ND, HOLWAY DA & CASE TJ (1999) Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. Biological Invasions 1:43-53.

SUAY-CANO VA, TINAUT A & SELFA J (2002) Las hormigas (Hymenoptera: Formicidae) asociadas a pulgones (Hemiptera: Aphididae) en la provincia de Valencia. Graellsia 58:21-37.

SUCKLING D, PECK R, MANNING L, STRINGER L, CAPPADONNA J & EL-SAYED A (2008) Pheromone Disruption of Argentine Ant Trail Integrity. Journal of Chemical Ecology 34:1602-1609.

SUCKLING D, PECK R, STRINGER L, SNOOK K & BANKO P (2010) Trail pheromone disruption of Argentine ant trail formation and foraging. Journal of Chemical Ecology 36:122-128.

TANAKA Y, NISHISHUE K, SUNAMURA E, SUZUKI S, SAKAMOTO H, FUKUMOTO T, TERAYAMA M & TATSUKI S (2009) Trail-following disruption in the invasive Argentine ant with a synthetic trail pheromone component (Z)-9-hexadecenal. Sociobiology 54:139-152.

THOMAS ML (2002) Nest site selection and longevity in the ponerine ant *Rhytidoponera metallica* (Hymenoptera, Formicidae). Insectes Sociaux 49:147-152.

TILLBERG CV, HOLWAY DA, LEBRUN EG & SUAREZ AV (2007) Trophic ecology of invasive Argentine ants in their native and introduced ranges. Proceedings of the National Academy of Sciences 104:20856-20861.

TINAUT A, FERNÁNDEZ-ESCUDERO I, RUANO F & CERDÀ X (1999) The Relationships of nest rock dimensions to reproductive success and nest permanence in a High-mountain ant, *Proformica longiseta* (Hymenoptera: Formicidae). Sociobiology 34:99-117.

TRONTTI K, ARON S & SUNDSTRÖM L (2006) The genetic population structure of the ant *Plagiolepis xene*-implications for genetic vulnerability of obligate social parasites. Conservation Genetics 7:241-250.

TSUTSUI ND, SUAREZ AV, HOLWAY DA & CASE TJ (2001) Relationships among native and introduced populations of the Argentine ant (*Linepithema humile*) and the source of introduced populations. Molecular Ecology 10:2151-2161.

VILÀ M, CASTRO P & GARCÍA-BERTHOU E (2008). Invasiones Biológicas. Ed.COMITÉ. CSIC. Capítulo 1:23.

VISSER D, WRIGHT MG & GILIOMEE JH (1996) The effect of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), on flower visiting insects of protea nitida mill. (proteaceae). African Entomology 4:85.

VITOUSEK PM, D'ANTONIO CM, LOOPE LL & WESTBROOKS R (1996) Biological invasions as global environmental change. American Scientist 84:468- 478.

VOGEL V, PEDERSEN JS, D'ETTORRE P, LEHMANN L & KELLER L (2009) Dynamics and genetic structure of Argentine ant supercolonies in their native range. Evolution 63:1627-1639.

WILD AL (2004) Taxonomy and Distribution of the Argentine Ant, *Linepithema humile* (Hymenoptera: Formicidae). Annals of the Entomological Society of America 97:1204-1215.

WILLIAMSON M & FITTER A (1996) The varying success of invaders. Ecology 77:1661-1666.

WITT ABR & GILIOMEE JH (1999) Soil-surface temperatures at which six species of ants (Hymenoptera: Formicidae) are active. African Entomology 7:161.

ANNEX

Publications derived from the Doctoral Thesis:

Here in, we detail the papers derived from the Doctoral Thesis, and at which point of the editorial process are each submitted manuscript is found. The Impact Factor (IF) corresponds to the latest update of 2011.

1. Diaz M, Abril S, Enríquez ML & Gómez C. Where to move when it gets cold: winter nesting sites attractive to the Argentine ant (Hymenoptera: Formicidae). Myrmecological News 18:51-58.

IF: 2.644. Rank 5/86 (Q1) in Entomology.

2. Abril S, Diaz M, Enríquez ML, Gómez C. (2012) More and bigger queens: a clue to the invasive success of the Argentine ant in natural habitats. Myrmecological News 18:19-24.

IF: 2.644. Rank 5/86 (Q1) in Entomology.

3. Diaz M, Abril S, Enríquez ML & Gómez C. Colony’s strategies of the Argentine ant in its native range: location of winter nesting sites and energy investment in queens. In preparation for Myrmecological News.

4. Diaz M, Abril S, Enríquez ML, Gómez C. Assessment of the Argentine ant invasion management by means of manual removal of winter nests in mixed cork oak-pine forests. Biological Invasions (second revision).

IF: 2.896. Rank 45/134 (Q2) in Ecology.

5. Diaz M, Enríquez ML, Abril S, Gómez C. Is the submissive ant *Plagiolepis pygmaea* affected by the presence of the Argentine ant in natural invaded areas? In preparation for Myrmecological News.