Invasion success of the Argentine ant: the role of native communities

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Invasion success of the Argentine ant: the role of native communities

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ABSTRACT

The Argentine ant (*Linepithema humile*) is an invasive species that has spread in Mediterranean-climate areas of the world. Once established in a new environment, the Argentine ant displaces the native ant community almost entirely, occupying the space very densely. However, although it has been described as a very successful invasive species, there are also examples of invasion failure.

In this dissertation I focus on these unsuccessful invasions, when the Argentine ant is not able to displace the native ant community and its abundance is not high. I focused on both the abiotic components of the environment, which can prevent or facilitate Argentine ant invasion of new localities, and the biotic factors that can influence the invader's performance or potentiate the effect of the abiotic components to prevent its success.

I used observational studies at a local scale (Doñana National Park, Spain; Dawson Los Monos Canyon, USA) and regional scales (coasts of Iberian Peninsula and Mediterranean islands), behavioral experiments (Dawson), long-term surveys (Doñana and Mediterranean islands), and data from literature of species co-occurring with the Argentine ant.

My results revealed that unsuccessful invasions are common and are not time-dependent, as the Argentine ant has been present in these localities for decades. This situation did not seem to be caused only by the lack of suitable abiotic conditions for the invader. Although the native ant community may not present biotic resistance in terms of species richness, species diversity, or community structure, I suggest that native ants with some life-history traits related to interspecific competition could be altering the invasion outcome when abiotic conditions are unsuitable for the invader. Field experiments showed that native species with these traits reduced foraging performance of the Argentine ant at baits, and I observed that these species co-occurred with the Argentine ant at a high frequency. Abiotic factors affected the spread of the Argentine ant at both regional and local scales. Besides human transportation, I found evidence that this invader might be using scavenger raptors to disperse at the local scale. At the regional scale probability of invasion was positively related to human presence and

proximity to already-invaded areas. Finally, on the coasts of Mediterranean islands, the Main supercolony expanded more than the Catalonian and Corsica supercolonies, which seems to be a result of arrival priority.

My dissertation highlights the importance of studying both biotic and abiotic factors in native ecosystems, as well as their interaction, in the long-term to modulate the success of the Argentine ant invasion.

RESUMEN

La hormiga argentina (*Linepithema humile*) es una especie invasora que se ha expandido por todo el mundo en zonas con clima mediterráneo. Una vez establecida en nuevo entorno, la hormiga argentina desplaza casi por completo a la comunidad nativa de hormigas, ocupando la zona a altas densidades. Sin embargo, a pesar de que ha sido descrita como una especie invasora muy exitosa, no siempre es el caso.

Esta tesis se centra en estas invasiones no exitosas, cuando la hormiga argentina no es capaz de desplazar completamente a la comunidad nativa de hormigas y su abundancia/densidad no es tan elevada. Para ello me he centrado tanto en los factores abióticos del medio, los cuales pueden prevenir o facilitar el éxito de la hormiga argentina para invadir nuevas localidades, como en los bióticos, que pueden influir en su rendimiento o potenciar el efecto de los factores abióticos para prevenir su éxito.

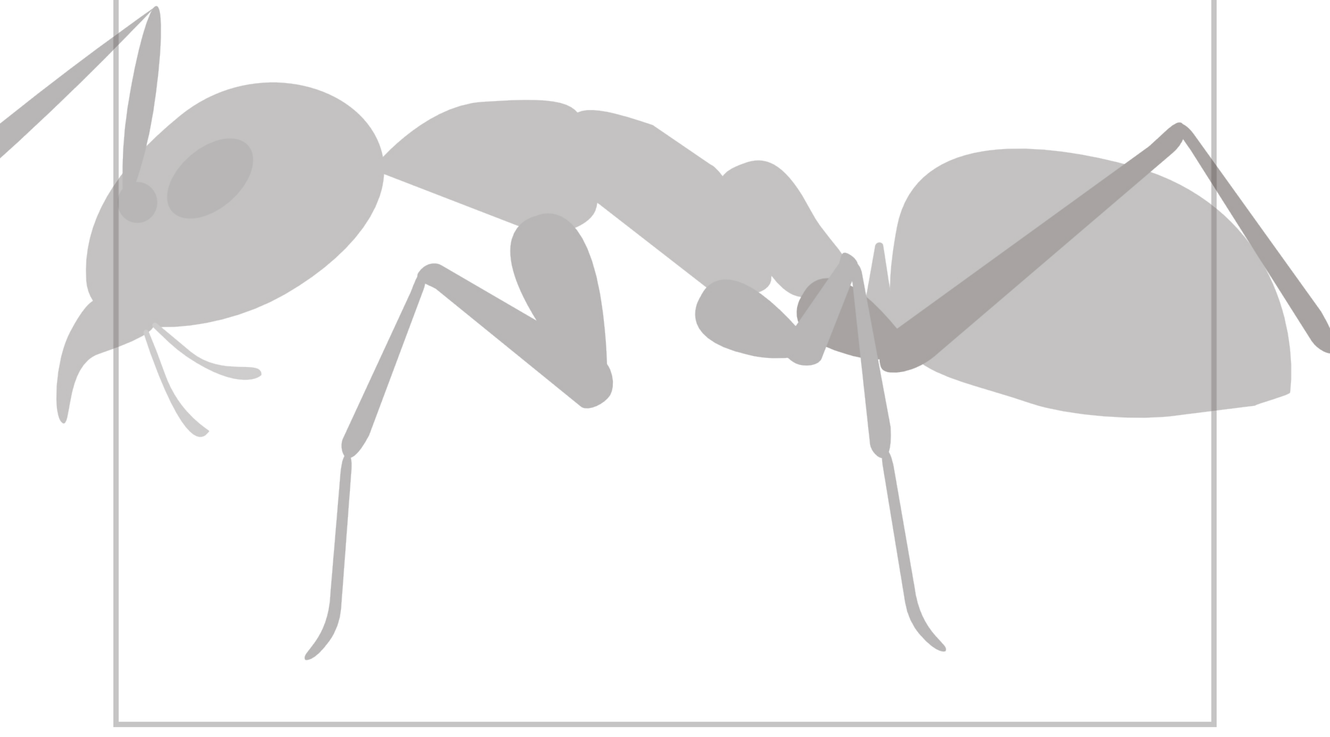
Para ello, he usado estudios observacionales a escala local (Parque Nacional de Doñana, España; Dawson Los Monos Canyon, EEUU) y regional (las costas de la Península Ibérica e islas Mediterráneas), experimentos comportamentales (Dawson), muestreos a lo largo de varias décadas (Doñana y las islas del Mediterráneo) y datos extraídos de la bibliografía sobre especies que co-ocurren con la hormiga argentina.

Mis resultados revelaron que las invasiones no exitosas de la hormiga Argentina son frecuentes y que no dependen del tiempo que lleve la zona invadida, ya que la hormiga argentina ha estado presente en estas zonas durante décadas. Este hecho no parece que sea debido solamente a la falta de condiciones abióticas adecuadas para la especie invasora. A pesar de que la comunidad nativa de hormigas no presenta resistencia biótica en términos de riqueza, diversidad o estructura de la comunidad nativa de especies, sugiero que aquellas hormigas nativas que poseen rasgos de vida relacionados con la competencia interespecífica podrían estar afectando al éxito de la invasión, especialmente cuando las condiciones abióticas no son adecuadas para la invasora. Experimentalmente, las especies nativas con estos rasgos redujeron la eficacia de forrajeo de la hormiga argentina en los cebos, y observé como estas especies co-ocurren frecuentemente con la

argentina en zonas invadidas de todo el mundo. Los factores abióticos afectaron a la expansión de la hormiga argentina tanto a escala local como regional. Además del transporte mediado por el hombre, encontré evidencias de que esta invasora puede estar usando rapaces carroñeras para dispersarse a escala local. A escala regional, la actividad humana y la distancia a zonas previamente invadidas, resultaron ser factores importantes en la invasión de nuevas zonas. Finalmente, en las costas de las islas del Mediterráneo, parece que la supercolonia europea se ha beneficiado de ser la primera en llegar para expandirse más que las supercolonias catalana o corsa.

En mi tesis subrayo la importancia de estudiar de forma paralela los factores bióticos y abióticos de los ecosistemas nativos y su interacción a lo largo del tiempo, ya que parece que modulan el éxito de la invasión de la hormiga argentina.

GENERAL INTRODUCTION AND GOALS



1. GENERAL INTRODUCTION

1.1. Biological invasions

Human development has progressed very quickly in recent centuries, making life more comfortable for our species and achieving goals never considered before. However, this progress has brought with it severe consequences for the environment, such as anthropogenic changes in ecosystems to develop cities, exploitation of resources, changes in land management for agriculture, waste in the ocean… One of the main consequences of human development has been the widespread introduction of non-native species all over the world (Seebens et al. 2017; Seebens et al. 2018). Introduction pathways have been diverse and can be unintentional, as in the case of numerous insect species that have ‘hitchhiked’ via human transportation systems (Rabitsch 2011). Over the past decades species have been more frequently introduced with a specific purpose for our own benefit, and in large numbers (Catford et al. 2018). Hunting, for example, has motivated the introduction of multiple game species of fishes, mammals and birds (Jeschke & Strayer 2006). Human preference for pets such as cats has made a great impact on biodiversity (Liberg 1984). Usage of ornamental plants has been described as the major cause of worldwide plant invasion (Wilson et al. 2012). Independent of the cause of their introduction, invasive species are one of the main reasons for biodiversity loss (Cox 1999; Simberloff et al. 2013; Bellard et al. 2016; Blackburn et al. 2019) as well as socio-economic impacts (Williamson 1996; Mack et al. 2000; Jeschke et al. 2014; Emiljanowicz 2017). Studies that help to understand how the process of invasion works are of great importance for future prevention and management of alien species.

1.2. The process of invasion and the success of the invader

A species must overcome several barriers in order to become invasive in a new environment (Blackburn et al. 2011; Fig. 1), and factors affecting success against these barriers may be different according to the stage of invasion (Leung et al. 2012). The first barrier is geographic, due to the need of a species to be transported to a new environment. In the case of intentional introductions, an additional captivity/cultivation barrier must be overcome. Once physical establishment has occurred, next stage’s barriers are survival and reproduction,

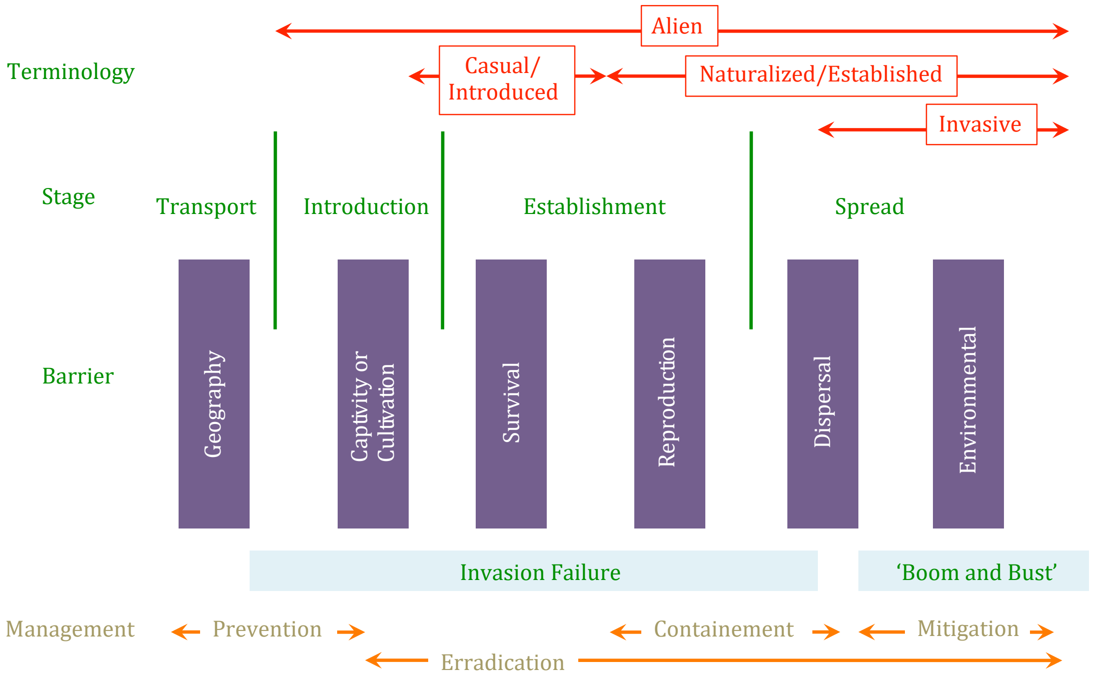


Figure 1. Stages and barriers that an alien species has to overcome in order to become invasive in a new environment, and the terminology for the species at each stage (modified from Blackburn et al. 2011).

to be able to establish in the ecosystem. The alien species, which until this stage was considered only introduced, becomes naturalized in the recipient ecosystem. At this point, dispersal represents the final barrier to a species becoming invasive. This barrier sets limits to the potential distribution range of the alien species, according to the characteristics of recipient ecosystems. It is in this framework, proposed by Blackburn et al. (2011), that my dissertation is structured. My purpose is to analyze what factors act as barriers to prevent spread of an invasive species.

Considering the high number of introduced species all over the world (more than 13 000 species; Ross N. Cuthbert, Pers. Comm.), it may seem a relatively easy process to succeed in invading a new ecosystem. But according to the Tens Rule, only 1 out of 10 introduced species becomes naturalized and only 1 out of 10 naturalized species becomes invasive (Williamson & Fitter 1996). The majority of studies about biological invasions focus on those that have succeeded (MacIsaac et al. 2011), but without an understanding of the mechanisms that make invasions fail we will never completely comprehend them (Nuñez & Medley 2011; Rodriguez-Cabal et al. 2013). Invasion failure can occur at any stage of the process, but early failures before the species have naturalized are more difficult to detect than failures in populations already established (Phillips et al. 2010). As Zenni & Nuñez (2013) proposed, long-term monitoring studies of populations are a good method to identify invasion failures, and this is one of the approaches that I follow in my dissertation. This approach allows observation of population dynamics (Lester & Gruber 2016) or the extent of the impacts of the invader (Jeschke et al. 2014), and has been used by several authors to analyze dynamics of invasive species (e. g. Nuñez & Medley 2011; Morris et al. 2013; Dyer et al. 2017; Ramirez et al. 2018). Tracking introductions through time would allow us to see differences between those environments where the invasion succeeded completely and those where the invader remains naturalized or even where invasion collapses. Thus, we will be able to measure how invaders are affected by both abiotic conditions and interactions with native species. Strayer et al. (2006) emphasized the importance of long-term monitoring studies because the impact of invaders can fluctuate and change over time. They illustrated their point with an example of how the red imported fire ant, *Solenopsis invicta*, invaded a region of Texas, decreasing arthropod diversity. However, when the area was re-sampled 12 years later, insect diversity had surpassed the pre-invasion level. If we observe only the early stage of the invasion we would conclude that *S. invicta* negatively impacts insect communities, while if we focus on the second sampling we would deduce that the presence of the invader favors native species. *Solenopsis invicta* was still the most abundant ant species in the second sampling, but not as abundant as it was during the initial phase of the invasion (Morrison 2002). Thus, monitoring invasive populations is an essential tool to determine not only the impact but also the expansion and success of the invaders.

The success of an invasive species may be estimated by monitoring the expansion rate of its distribution. However, there is an important bias in the data due to the lack of reported naturalizations/invasion failures (Diez et al. 2009; Rodriguez-Cabal et al. 2013). Nuñez and Medley (2011) studied the success of *Pinus* populations worldwide and stated that if they had not analyzed the failed invasions they would have overlooked the importance of biotic interactions in the recipient environment as a barrier to *Pinus* invasion. If we could analyze not only positive data but also the negative (failures at some point of the invasion process), we could have a more accurate measurement of the success of an invader. This approach requires tracking invasive populations through time.

1.3. Factors affecting successful invasions

Among the most common factors preventing the establishment or spread of introduced species are biotic and abiotic resistances (e.g. Zenni & Nuñez 2013). Depending on the species of interest, variability in abiotic factors like soil type (Wang et al. 2017), temperature (Penk et al. 2016), disturbance (Kneitel & Perrault 2006), and humidity (Menke & Holway 2006) can influence the success or failure of the introduced species. Although species vary in their abiotic tolerance ranges (Zhan et al. 2015), establishment in a foreign environment often requires climate matching to the native range of the invader (Nuñez & Medley 2011). Global climate change is going to alter invasive species distributions in addition to their performance and impacts on native communities (Dukes & Mooney 1999; Bradley et al. 2010; Medina-Villar et al. 2020) Although distributions may be reduced for certain invasive taxa such as amphibians or birds (Corn 2005; D'Amen et al. 2011), arthropods should benefit from this increase in temperatures and broadly expand their range of invasions (Ward & Masters 2007; Bellard et al. 2013). Although matching climate conditions are very relevant, alien species can survive in suboptimal conditions, where their populations may be limited in size or performance (Ricciardi et al. 2013). Thus, invasive species coping with unfavorable abiotic conditions may be more vulnerable to pressure by other factors, such as the biotic resistance of the recipient ecosystem.

The biotic resistance hypothesis states that native ecosystems that are richer in species can resist invasion through factors like predation, competition, aggression, disease or parasitism (Elton 1958). These factors could be acting in different ways and at different grades depending on the stage of the invasion process. In their meta-analysis on plant invasions, Levine et al. (2004) showed how most studies of biotic resistance are based on populations that have already passed the naturalization period. Even knowing this bias, Levine et al. (2004) showed that biotic resistance limited the abundance of an established invader. Biotic resistance from native ecosystems can manifest in several ways, corresponding to specific hypotheses (Catford et al. 2009; Riccciardi et al. 2013).

The empty niche hypothesis states that higher species richness results in fewer available niches in the community, reducing the likelihood of invasion. (Stachowicz et al. 1999; Naeem et al. 2000; Hierro et al. 2005; Bufford et al. 2016; Liew et al. 2016; Beauri et al. 2020). Another hypothesis linked to biotic resistance is the generalist-specialist hypothesis, which states that if predators in a recipient ecosystem are generalists, then invaders are less likely to succeed due to the probability of being consumed (Catford et al. 2009, Sax et al. 2007). This is closely related to the exotic prey naïveté hypothesis and the increased susceptibility hypothesis, which postulate that new invaders experience higher levels of predation from predators unknown to them (Colautti et al. 2004; Catford et al. 2009; Li et al. 2011; Wanger et al. 2011). Thus, invasions are more likely to fail in communities with high species richness and/or a predominance of generalist predators.

Biotic resistance could also depend on the identity of the native species of the recipient community, or on specific life-history traits they may possess. According to the limiting similarity theory, native species that possess traits that overlap with those of the invader will be more capable of competing with it for key resources, jeopardizing the invader's establishment (Funk et al. 2008) or at least reducing the degree of its success. But, what is the degree of effectiveness of each trait to face invasive species? Most studies about biological invasions focus on the traits of invasive species (Sans et al. 2004; Lloret et al. 2005; Jeschke & Strayer 2006; Wittenborn & Jeschke 2011; Allen et al. 2017; Swart et al. 2018) to understand the mechanisms that make them successful invaders. However, predicting the vulnerability of specific native ecosystems will also require an understanding of the traits that enable native species to repel the invasion.

Trait overlap with native species could also end up in modification of specific traits of the invader to avoid competition, for example with trait plasticity (Funk et al. 2008). Rapid adaptation through epigenetic changes confers advantages to invasive species under the pressure of novel conditions (Ricciardi et al. 2017). Several studies have shown how invaders can adjust to the conditions they face in new environments. Introduced wasps like *Vespula vulgaris* or *V. pensylvanica* can outcompete native species through behavioral plasticity (Wilson et al. 2009; Grangier & Lester 2015). Traits of invasive *Acer* species related to germination and leaf durability show variation according to the moisture availability of the environment they are invading (Zenni et al. 2014). Females of invasive populations of round goby *Neogobius melanostomus* modify several reproductive and morphological traits during colonization in order to succeed (Hôrková & Kovác 2014). Therefore, traits of invasive species should not be studied through snapshots, because they may express plasticity to adapt to diverse conditions in space and time. By identifying which traits of native species provide a basis for competitive biotic resistance, we may predict which traits of the invader are under selection for plasticity.

1.4. The Argentine ant as an invader

I focus my dissertation on the invasive Argentine ant (*Linepithema humile*), a very harmful invader that has spread all over the world in areas with Mediterranean-like climate (Suarez et al. 2001). As the second-most studied invasive species, after the zebra mussel (Pyšek et al. 2008), numerous studies are available to understand its biology, life-history traits, and mechanisms of invasion. Native to Argentina, Brazil, Uruguay and Paraguay, the Argentine ant was unintentionally spread through commerce routes in 19th century Europe (Martins 1907; Wetterer et al. 2009). It has been included as one of the 100 world’s worst invaders by Lowe et al. (2000), due to its great impact on the ecosystems it invades. Once installed in a new environment, the Argentine ant is able to displace the native ant community almost entirely, with the possible exception of some very small, cryptic and/or evasive species like *Plagiolepis pygmaea* (Abril & Gomez 2009). Ants are not the only taxonomic group affected by the invasion. Arthropod communities in general suffer important alterations in the presence of the Argentine ant, as demonstrated in Northern California, Hawaii, and Australia (Holway 1998; Rowles & O’Dowd 2009a; Krushelnycky & Gillespie 2010). Small vertebrates can be also threatened by this invasive species, including amphibians (Alvarez-Blanco et al. 2017; in press b), reptiles (Suarez & Case 2002) and birds (Boieiro et al. 2018; Alvarez-Blanco et al. in press a). By affecting all these species, the Argentine ant's invasion provokes indirect alterations such as lost seed dispersal (Rowles & O’Dowd 2009b) or reduced plant growth and pollination derived of tending honeydew-producing hemipterans (LeVan & Holway 2005; Nygard et al. 2008).

The Argentine ant’s invasion success is a product of several life-history traits, common to the most invasive ant species, which make them superior competitors (Passera 1994). The presence of multiple queens in the same nest (polygyny), together with the workers' relatively small size, promotes the transport of small propagules (without humans noticing) it or its dispersal by budding. A generalist diet also facilitates its establishment in a wide range of environments. Probably the most important trait for invasion success is a special type of social organization called unicoloniality. This consists of the ability to form large colonies of workers (supercolonies) lacking intraspecific aggression, moving freely among nests that can be even thousands of kilometers apart (Giraud et al. 2002). This behavior facilitates invasion, enabling workers to collaborate against native species instead of competing with neighboring nests of other Argentine ants. At least 15 different supercolonies have been described worldwide (Vogel et al. 2010), the largest of which is known as the Main European. This supercolony, while present on all continents, occurs along more than 6 000 km in Southeastern Europe alone (Giraud et al. 2002), forming the largest cooperative unit ever documented (van Wilgenburg et al. 2010).

Although the Argentine ant is usually reported as a very successful species, it is not always as successful as expected (Way et al. 1997; Thomas & Holway 2005; Wetterer et al. 2006). These unsuccessful invasions can persist over time (Mothapo & Wossler 2014) and can provide very interesting information about the characteristics of the recipient ecosystems that may be preventing the invader from succeeding completely. Although the term ‘successful invasion’ could be interpreted in different ways according to the invasive species we are studying and the degree of success we want to imply. In this dissertation I consider the Argentine ant fully successful when it has displaced the native ant community. According to this, I categorized an area as successfully invaded if the Argentine ant was present in very large numbers and native ant species were displaced, or if a maximum of three cryptic subordinate species were coexisting with the Argentine ant by avoidance. When the Argentine ant was present in lower numbers and was part of the ant community among the rest of native ant species, I categorized those areas as unsuccessfully invaded.

2. GOALS

This dissertation studies the invasibility of the native communities and their vulnerability to invasion by the Argentine ant, analyzing what factors influence the spread and success of this invader. I focused on the abiotic components of the environment, which can prevent or facilitate Argentine ant success at invading some localities, and the biotic factors that can influence invader's performance or potentiate the effect of the abiotic components. I tested the biotic resistance and empty niche hypotheses, combining field samplings and experiments at local or regional scales with literature review on native species' life history traits. I tested the potential behavioral plasticity of the invader using lab experiments. Finally, I explored the effect of abiotic factors using historical and geographic data. Specific goals for each chapter are described below.

Chapter I: Long-term spread of Argentine ant European supercolonies in three Mediterranean islands

The main goal in this chapter was to analyze environmental and spatial factors that may be affecting the spread and success of the Argentine ant along the coasts of Corsica, Ibiza and Formentera. All three known supercolonies in Europe are present in these three islands. By implementing two rounds of sampling 9-12 years apart, we are able to study the temporal progression of the Argentine ant invasion in this region. Specifically, I study the Main, the Corsican and the Catalonian supercolonies. The specific goals were:

(a) Analyze what environmental and spatial factors could be affecting the likelihood of invasion by the Argentine ant and its success. I compared the characteristics of invaded vs. uninvaded beaches, and within the invaded beaches I compared those successfully invaded with those unsuccessfully invaded.

(b) Test whether the differential spread of the three supercolonies is caused by differences in their response to the environmental or spatial factors or to the history of invasion.

Chapter II: Humans and scavenging raptors facilitate Argentine ant invasion in Doñana National Park: no counter-effect of biotic resistance

I aimed to assess the relative roles of abiotic factors (environmental and spatial) and biotic resistance of the native community in the spread of the Argentine ant at Doñana National Park (Spain). As in the previous chapter, I have a temporal scale (data from 1992, 2000, 2016) that allows me to study the progress of the invasion, but in this case at a local scale. The goals for this chapter are:

(a) Test the effect of biotic resistance, specifically whether native species richness, proportion of native dominant species, and native ant community structure at uninvaded plots in 1992 could be limiting future invasion by the Argentine ant.

(b) Test what environmental and spatial factors drive the local spread of the Argentine ant in a matrix of favorable and unfavorable habitats, including the role of humans (roads or paths) and scavenger raptors (breeding sites) as dispersal vectors of Argentine ant propagules.

Chapter III: Can the success of the invasion be determined by biotic resistance and presence of empty niches in native communities?

The main objective in this chapter is to analyze whether the biotic resistance hypothesis and/or the empty niche hypothesis explain unsuccessful invasions by the Argentine ant. I focus specifically on successfully and unsuccessfully invaded areas of the Iberian Peninsula and Balearic islands. I used a paired design with invaded and uninvaded control plots following a space-for time-substitution approach. The specific goals were:

(a) Test the biotic resistance hypothesis: whether high richness, diversity, relative proportion of dominant native ant species, or a different native community structure could prevent the Argentine ant from successfully invading.

(b) Test the empty niche hypothesis: whether the presence of empty niches could be facilitating the success of the invasion.

(c) Study how behavioral plasticity in aggressiveness may influence success of the invasion by the Argentine ant. That is, test whether changes in intraspecific abilities of the Argentine ant could be causing the failure of invasion.

Chapter IV: Competitive interactions between native species and the invasive Argentine ant according to behavioral dominance and foraging strategy

In this chapter, I studied the role of two life history traits of native species in reducing foraging ability of the Argentine ant. I performed a field experiment in Dawson Los Monos Canyon Reserve in San Diego County (USA) where the Argentine ant co-occurs with native species (very likely because of dry conditions). Experiments at this local scale were complemented by a literature review on other native species co-occurring with the Argentine ant. Two specific objectives for this section are:

(a) Test whether Argentine ant foraging activity could be reduced by competition with native ant species presenting none, one, or both of the life-history traits selected (recruitment at foraging and behavioral dominance). I performed a reciprocal short-term removal experiment using baits, and characterized bait monopolization by all species.

(b) Quantify whether the frequency of two life history traits (mass recruitment at foraging and behavioral dominance, related to exploitative and interference competition) were associated with the co-occurrence of the Argentine ant with native species (as a measure for unsuccessful invasions).

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LONG-TERM SPREAD OF ARGENTINE ANT EUROPEAN SUPERCOLONIES IN THREE MEDITERRANEAN ISLANDS

Long-term spread of Argentine ant European supercolonies in three Mediterranean islands

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ABSTRACT

The Argentine ant is an invasive species that has spread all over the world, and is organized in several known different supercolonies. However, while there are many studies about factors promoting the expansion of the species, little is known about the factors affecting the differential expansion and success of the different supercolonies. We study what environmental and spatial variables were affecting the invasion of the Argentine ant in three islands of the Mediterranean Sea (Corsica, Ibiza and Formentera) and in the three European supercolonies that inhabit them (Main European, Corsican, and Catalonian). For that, we used data from two samplings spaced 9 years in the case of Corsica and 12 in the case of Ibiza and Formentera, and historical data of first detection dates and locations in islands of Southwestern Europe. Along the coast of the three islands, we sampled each beach to detect presence of the Argentine ant and we identified the supercolony. The Argentine ant expanded in the three islands. Although the three supercolonies maintained the invasion in the same locations and expanded to new locations, the highest expansion was for the Main supercolony in Ibiza and Formentera (even if the Catalonian was the first detected in Formentera) but for the Corsican supercolony in Corsica (even if the main supercolony seemed to have the first detected in Corsica). Interestingly, the invasions were not always successful: the Argentine ant co-occurred during 12 years with native species in one third of the beaches of Ibiza and Formentera. Human presence affected the likeliness of a beach to be invaded in Ibiza and Formentera. In Corsica, beaches that were invaded, or got invaded during our study, were the ones with lower distance to already invaded beaches, suggesting the importance of secondary introductions in the local expansion of the Argentine ant. Our findings help to understand the dynamics of invasions of the Argentine ant and its different supercolonies. Because the expansion of the Argentine ant in Europe is a fact, long-term studies at many invaded sites are of great importance in order to obtain general patterns of the spread of this global invader.

KEY WORDS

Linepithema humile, biological invasions, supercolonies, biotic resistance, invasion pathways

1. INTRODUCTION

Biological invasions are one of the major threats to native communities, leading to a loss of biodiversity (Cox 1999; Simberloff et al. 2013; Bellard et al. 2016; Blackburn et al. 2019). Humans have facilitated the entrance of new species in areas where geographical barriers prevented it, like the case of islands (Williamson 1996). Islands are characterized by low species richness and less complex food webs but at the same time, due to their isolation, they have higher rates of endemism (Pimm 1996; Naughton et al. 2019). These characteristics make them very vulnerable to invasions (Bellard et al. 2017, according to the Eltonian theory of biotic resistance, Elton 1958), with the loss of species, even before they have been discovered (Lees & Pimm 2005). Native communities in islands are shaped by the theory of island biogeography (MacArthur & Wilson 1967), where isolation and island size are crucial for richness and diversity. Although these two factors could reduce the probability of invasive species reaching the island, human movements such as international trade industry or tourism activity, facilitates their arrival (Courchamp et al. 2017; Seebens et al. 2017). So, knowing what other factors can influence this expansion of alien species in islands and their successful establishment is vital to work on the preservation of these fragile and valuable ecosystems.

Several ant species are considered among the world worst alien species (Lowe 2000; Invasive Species Specialist Group's Global Invasive Species Database, http://www.iucngisd.org/gisd). This is the case of the Argentine ant (*Linepithema humile*) whose success is due to a series of life-history traits that make it a very efficient invader. One of the most important ones is the unicoloniality, that is a kind of social organization where nestmates do not show aggression among nests that could be thousands of kilometers apart (Giraud et al. 2002). This confers an ecological advantage to the Argentine ant because intraspecific aggression is changed into collaboration among nests for the success of the supercolony. This way, the species is able to locally eradicate native ant species from the ecosystems they invade. Unicoloniality in the Argentine ant leads to the appearance of different supercolonies in the world, with workers showing inter-specific aggression among supercolonies (Vogel et al. 2010). Vogel et al. (2010) described at least 15 supercolonies around the world. The largest supercolony is the so-called, European Main supercolony (hereafter, Main supercolony), which ranges over 6 000 km in Southwestern Europe (Giraud et al. 2002) and spreads all over the world, forming the largest cooperative unit ever recorded (van Wilgenburg et al. 2010). In Europe, three different mutually aggressive supercolonies are established. Besides the Main supercolony, the Catalonian supercolony spreads mainly over the East coast of the Iberian Peninsula and the Balearic Islands (Gomez & Espadaler 2005) although it seems to have been recently detected in North France and Southwest Spain (Charrier et al. 2020 cited it in the city of Nantes France) , and personal observations for a citation at Coria del Río, Spain); while the Corsican supercolony is present in Corsica and the South coast of France (Blight et al. 2010b, 2012; Berville et al. 2013).

The Argentine ant has successfully spread across many zones of the globe with Mediterranean and sub-tropical climates, through the intensification of human activities (Suarez et al 2001). It has preference for habitats with mild temperatures and moist, with dense or patchy vegetation to help to provide these conditions (Hartley et al. 2006; Angulo et al. 2011; Roura-Pascual et al. 2011; Castro-Cobo et al. 2019). As most invasive ants, its nesting sites plasticity allows them to invade easily human constructions (Lee 2002), like settlements or boats docks (Carpintero et al. 2003, Rizali et al. 2010) where it can find very stable climate and a constant source of food and water to establish populations. These abiotic requirements, together with ports of entries as the main invasion pathway (Corin et al. 2007; Bertelsmeier et al. 2017), shape its distribution and expansion along coastal areas in Southwestern Europe (Espadaler & Gomez 2003; Gomez & Espadaler 2006; Blight et al 2009; Wetterer et al. 2009).

As it has small size workers and high number of queens at nests, small propagules can be transport by humans unnoticed. Thus, in places with high rates of human frequency, like very touristic areas such as islands, the Argentine ant can be easily transported and established if it finds suitable conditions. More locally, the Argentine ant has been able to spread with the help of other dispersal strategies. Argentine ant populations are able to progress 150 m/year the invasion front just by budding (Suarez et al. 2001). Human paths and corpses of raptors' prey dispersal to cross unsuitable places have been also described as local dispersal modes for this invader (Castro-Cobo et al. 2019).

Looking at the expansion of the Argentine ant in the world, it is clear that the spread of the Main supercolony is broader and faster than the spread of the other ones (Vogel et al. 2010; van Wilgenburg et al 2010), so there may be some factors favoring this uneven expansion. However, there is no information about which factors could cause the differential expansion of the Main supercolony. It could be related to the history of the invasion of the Argentine ant (Wetterer et al. 2009). If the first arrivals to the invasive range pertained to the Main supercolony, secondary introductions, which are common in invasive ants (Bertelesmeier et al. 2017, 2018), could have fueled this supercolony against the others. However, it could be also that some other factors, more related to the suitability of the environment could explain the differences.

In this study, we aim to analyze the factors affecting the spread and the success of the Argentine ant invasion, specifically differentiating between the three different supercolonies present along the Southwestern Mediterranean coast. We selected three islands: Corsica, where the Corsican and Main supercolonies are present, and Ibiza and Formentera (the Pityusic Islands), where the Catalonian and the Main supercolonies are present. We used a temporal approach, recording the spread of the invasion with a lag of 9 and 12 years: we compared the invasion status along islands' coast in Corsica between 1999 and 2007 and in the Pityusic Islands between 2004 and 2016. First, all the beaches along the coast were visited in order to record presence and relative abundance of the Argentine ant. Then, we obtained spatial factors related to human-driven dispersal and environmental factors related to habitat characteristics, in order to test whether these factors affected to the invasion process in terms of (i) presence of the Argentine ant (current distribution of invaded beaches), (ii) success of the invasion, (iii) progress of the invasion (i.e. the invasion of new beaches) through time and (iv) differences among the three supercolonies. We hypothesized first, that locations highly disturbed by humans, with more availability of dense vegetation (due to its preference for habitats with mild temperatures and moist; Hartley et al. 2006; Menke et al. 2007), closer to other already invaded areas, and/or more accessible for humans would be the ones with more probability of being invaded by the Argentine ant, and also the invasion to be more successful. Second, we would not expect that any of the environmental variables could be facilitating more the spread of the Main supercolony over the other two smaller ones, but that the higher expansion of the Main supercolony would respond more likely to the invasion history. We tested this last prediction using historical data on first detection dates and locations of the Argentine ant invasion for 19 islands in Southwestern Europe.

2. MATERIAL AND METHODS

2.1. Study area

Fieldwork was carried in three Mediterranean islands: Corsica, Ibiza and Formentera (Fig. 1). Ibiza and Formentera are two Spanish islands that belong to the Balearic archipelago, located to the East of the Iberian Peninsula. Corsica is a French island located halfway between the Italian and the French coast.

They are characterized by Mediterranean-like climate, with mean annual temperature in these islands are around 15-17ºC (Morey & Ruiz-Perez 2008; Mouillot et al. 2008). Ibiza and Formentera consist in a mixture of arid areas with woodlands of *Pinus halepensis* and *Juniperus phoenicea* or mixed formations of both (Morey & Ruiz-Perez 2008). Corsica's coastal vegetation consist in *Erica arborea* as main native species with introduced *Quercus ilex* and *Pinus halepensis* (Reille 1992). Human population considerably increases in summer due to the high pressure of tourism in the three islands. The daily number of tourists in august in Corsica can reach more than 430 000 individuals which the double of inhabitants. The total number of tourists per year is around 7.7 million, it reached 8.2 million in 2017 (Genovese-Bolleyn et al 2018). In 2018 the number of tourists in Ibiza and Formentera was more than 3 million tourists (AETIB 2018).

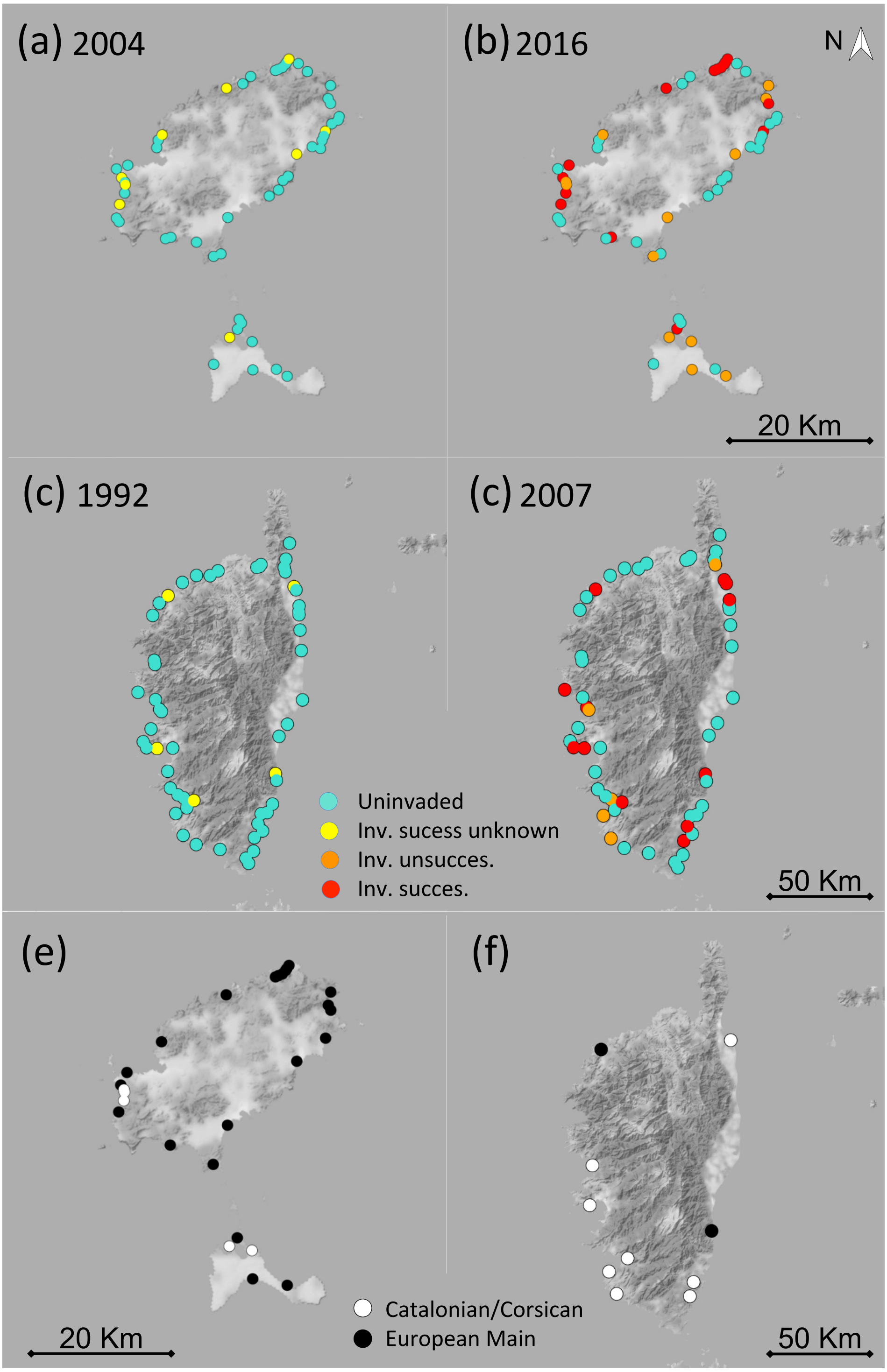

Figure 1. Invasion of the Argentine ant in the three Mediterranean Islands. Invasion status of sampled beaches in Ibiza and Formentera in 2004 (a) and 2016 (b), and in Corsica in 1992 (c) and 2007 (d). Argentine ant supercolonies are shown in Ibiza and Formentera in 2016 (e) and in Corsica in 2007 (f).

2.2. Sampling Argentine ant populations and supercolonies

The three supercolonies of Argentine ant are present in these islands: the Catalonian (in Ibiza and Formentera), the Corsican (in Corsica) and the Main (in the three of them). Ibiza and Formentera were first sampled in 2004 by Gomez & Espadaler (2005), when all the accessible coast of the islands was examined to locate populations of Argentine ant. The ants were sampled by searching for them by eye during at least 30 minutes in the suitable parts of the beach, such as car parks, streets or avenues, vegetation (natural or artificial), buildings or other human constructions. When the invader was found, they performed one-on-one aggression tests with workers from both the Main and the Catalonian supercolonies to determine the identity of the local population. Five replicates for each of the two combinations were carried out. When no aggression was recorded, the local population was assigned to the supercolony of its opponent (Gomez & Espadaler 2005).

In year 2016, we repeated the sampling to check the expansion of the invasion of the two supercolonies. A total of 56 beaches were surveyed. In this case, besides recording the presence or absence of the Argentine ant, we also recorded the native ant species that we observed during the prospection, in order to have an index of invasion success as explained below.

Corsica was sampled in 2007 and 2008 by Blight et al. (2009). Due to the size of the island, which is considerably larger than the other two, it was not possible to conduct a comprehensive sampling of the coast. A total of 59 beaches were surveyed. At each site, ground was inspected, searching under stones or dead woods during 20 minutes scan. During the scan several observations were made, separated by at least 5m, and we recorded not only the number of observations with Argentine ant but also the number of observations with native ants, in order to obtain an index of invasion success as explained below. Information about the identity of the supercolony was extracted from Blight et al. 2012 for some of the sites. A previous sampling was made by Casevitz-Weulersse & Brun in 1999, although it was not as exhaustive as the one performed by Blight et al. (2009) in 2007-08, but we extracted the information from the sampling points of this article for some of the analysis.

2.3. Categorization of the beaches

From the surveys in these three islands, we considered first the invasion of the beach: if the beach had presence of Argentine ant when last sampled (2016 for Ibiza and Formentera, 2007-08 for Corsica) we categorized it as invaded; it was categorized as uninvaded if there was no presence of the Argentine ant (Fig. 1).

Second, each beach that was invaded was categorized by the success of the Argentine ant invasion. The success was estimated by the presence or absence of native ants together with the Argentine ant abundance. In general, a successful invasion was defined when the Argentine ant was the only species of the invaded community and was at high abundance, and an unsuccessful invasion was when the Argentine ant was co-occurring with native ant species, at low, similar abundance than the native ants. Because the sampling on Balearic Islands was slightly different from the sampling in Corsica, the categorization was also different. In the Balearic Islands a successful invasion was assigned when the Argentine ant was the unique species found in the beach and in large numbers, or with a maximum of three native species in very low numbers. Unsuccessful invasion was assigned when the Argentine ant co-occurred with more than 3 native species and it was present in low numbers. Thus, we established a binary variable for successful vs. unsuccessful invasion (Fig. 1).

In Corsica, we estimated the percentage of sampled points at each beach in which the Argentina ant was present. That is, when a site was scored as invaded, we calculated an invasion success index that was the ratio between the number of Argentine ant observations that were separated by at least 5 m and the total number of observations. Although a successful invasion could be considered when there were more than 70% of points with presence of Argentine ant, we preferred to use the percentage itself, a continuous variable, as a proxy for the success of invasion (Fig. 1).

Third, we had information of where the three supercolonies were located (Fig. 1). In the Balearic Islands, we were able to assess the supercolony to which the Argentine ant belonged in both samplings (2004 and 2016). In Corsica, as we did not have information from aggression test to identify the supercolony at each invaded site, we extracted the information from literature. Blight et al. (2012) studied the supercolonies at Corsica and 10 of their study sites coincided with ours, so we could extract the information for these localities.

Finally, we had information about how new invaded beaches appeared in each island in the second survey (Fig. 1). Thus, we could analyze what factors could affect the invasion of new beaches along time. In Ibiza and Formentera, using the beaches that were uninvaded in 2004, we compared the ones that remained uninvaded in 2004 with the ones that changed their status to invaded in 2016. In Corsica, we recurred to literature again. Casewitz-Weulersse & Brun (1999) surveyed the island and published a list of the beaches that were invaded. We used this information to determine if the beaches invaded in 2008 were new invasions or if they were already invaded in 1999.

2.4. Environmental and spatial variables affecting the invasion

In order to study what factors can affect to the expansion of the invasion in these islands, its success in invaded locations and the differences between supercolonies, we measured the following environmental and spatial variables:

For the spatial variables we measured the distance to the nearest invaded beach, as a potential invasion source, and to the closest building. We used the tool “Ruler” in Google Earth Pro (version 7.3.2.5776, Google Earth Pro 2019) to calculate distances, taking the middle point of the beach coast as reference. For the distance to the nearest invaded beach, in the cases when we knew the identity of the supercolonies, we measured the distance to the nearest beach invaded in the first sampling that belonged to the same supercolony. Details of which analysis used the variable ‘distance to the nearest invaded beach of the same supercolony’ are in Table SM_1. We also measured the length of the beach with the tool “Add path” in Google Earth Pro (version 7.3.2.5776, Google Earth Pro 2019), at 3 km of eye view, to calculate the total length of the coastline.

For the environmental variables, we wanted to obtain information about two main factors that could affect the Argentine ant invasion: vegetation types (as a proxy for soil moisture and temperature, Angulo et al. 2011; Menke et al. 2007), and human constructions. Using geographic information system (ArcGIS, version 10.5) we create a grid over each sampled point of 210 m^2, formed by pixels of 30x30 m^2, using the tool *Create Fishnet*. Thus, we obtained a square of 7x7 pixels per sample point that we exported to kml to work on Google Earth Pro (version

7.3.2.5776, Google Earth Pro 2019). We counted the number of pixels of sea, beach, scarce vegetation, dense vegetation, human construction and naked soil we had, to get a description of the surrounding habitat of the sampled areas (Fig. 2). As sea pixels do not provided us any valuable information, we removed them from the final count and recalculate the other environmental variables proportionally to the removal of sea pixels. We also considered other factors that could be related to the Argentine ant survival (when first locally introduced into a beach) or to human intensity (number of visitors to the beach), such as the presence of a bar, a pier/port, the type of trail that leads to the area (road/path), or the main material forming the coast (sand/rocks).

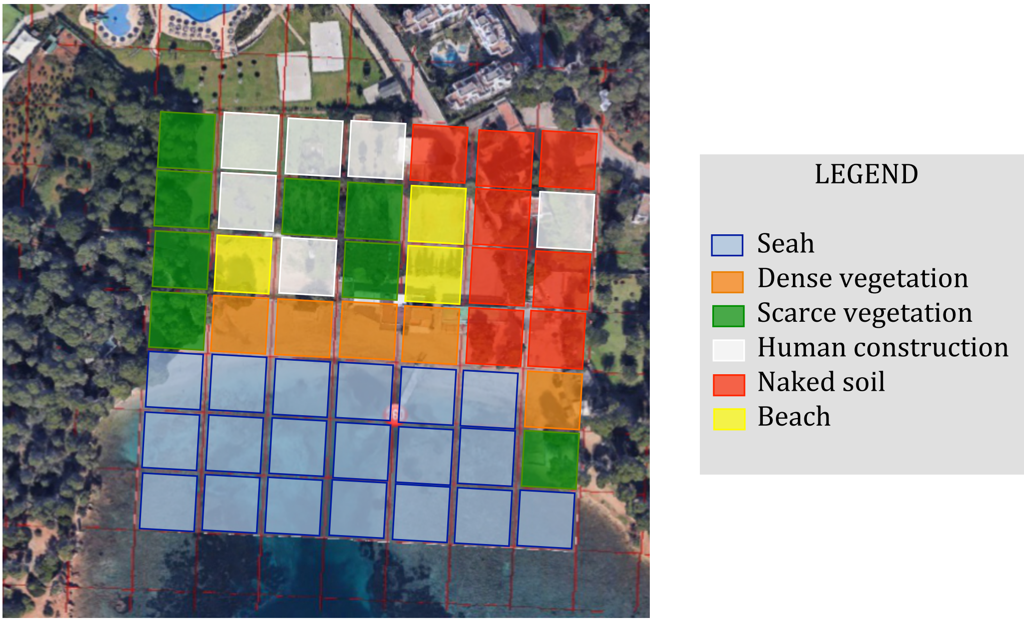

Figure 2. Example of environmental variables measurements of a beach in Ibiza (Cala Pada). The red grid used as well as the different categories each grid could be assigned to are shown.

2.5. Data analysis

All the analyses in this study were conducted in R (R Studio version 1.0.136, RStudio Team 2016). Because dependent variables analyzed were different for each analysis described below, sampling size was also different in each analysis (Table 1).

To reduce the number of environmental variables we conducted a Principal Component Analysis using the *princomp* function (stats package, R Core Team

Table 1. Number of beaches per island used in each of the analyses performed. Analyses represent invasion status (invaded vs. uninvaded), invasion success (successful vs. unsuccessful invasion), differences between supercolonies (Main vs. Catalonian or Corsican) and change of status (change from uninvaded to invaded vs. no change). Categories marked in bold are the ones modeled (models with binomial link distribution).

	Invasion		**Success**		**Supercolony**		**Change of status**	
	Yes	No	**Yes**	No	**Main**	Cat/Cor	**Yes**	No
Ibiza-Form.	28	28	16	12	23	5	19	28
Corsica	19	40	14	5	2	8	14	40

2017). We selected the principal components (herein, dimensions) that explained >10 % of the variance (Table 2). We obtained the contributions of the environmental variables to each dimension (Table 2), and finally we extracted the values of each selected dimension to each sampled location, and included them in the analysis as independent variables.

In order to analyze what factors affected to the invasion of the Argentine ant, we performed four different generalized lineal models using the *glm* function (package stats, R Core Team 2015) for each of the islands data sets: Ibiza and Formentera together, and Corsica. Dependent variables of the four models were: (i) the status of the invasion (if the beaches were invaded when last sampled or not); (ii) the success of those ones invaded (successful or unsuccessful invasion); (iii) which supercolony is the one present in the invasion (Main or Catalonian for Ibiza and Formentera, and Main and Corsican for Corsica); and (iv) the change of invasion status over time (the beach remained uninvaded or it changed its status to invaded).

The four models had a binomial link distribution, and each model comprised as independent variables the previous spatial parameters and the selected components from the PCA (in Table 2). For each model, the category that was modeled by the binomial distribution is marked with bold in Table 1. The complete model was evaluated, except in the case of the analysis between supercolonies in Corsica. In this case, because the subset analyzed had a low sample size (Table 1), we used a forward stepwise regression.

Table 2. Contribution of the variables included in the PCA to each one of the dimensions (D.1, D.2 and D.3) selected for each analysis in (a) Ibiza and Formentera and (b) Corsica. Contribution is represented by a percentage and the sign represents the direction of the relationship of each variable with the given dimension. White cells represent values lower than 1%. Lighter grey are for values between 1 and 30%. Darkest grey we represented values higher that 30%. Percentage of explained variance for each PC is given in the last row (% Variance).

(a)	Invasion			Success		Supercolony		Change of status		
IBI-FORM	D.1	D.2	D3	D.1	D.2	D.1	D.2	D.1	D.2	D.3
Bar										
Access										
Material										
Dock										
Beach	1 +		14 -					1 -		14 +
Dense veg.	**85 -**	13 -		**77 -**	23 +	**77 -**	23 +	**77 +**	18 -	2 +
Scarce veg.		16 +	**70 +**						18 +	**68 -**
Naked soil									2 +	
Construction	14 +	**70 -**	14 +	22 +	**74 +**	22 +	**74 +**	21 -	**61 -**	15 +
% Variance	**50**	31	11	**63**	27	**63**	27	**47**	32	13

(b)	Invasion			Success			Supercolony			Change of status		
CORSICA	D.1	D.2	D.3	D.1	D.2	D.3	D.1	D.2	D.3	D.1	D.2	D.3
Bar									1 +			
Access												
Material												
Dock									2 -			
Beach		**69 -**			15 -	14 -		8 -	**38 +**		**71 -**	7 -
Dense veg.	3 -	6 +	**78 -**	3 -	**78 +**	11 -	14 -	**76 +**	3 +	1 -		**70 +**
Scarce veg.	**51 -**	8 +	18 +	**69 -**		25 +	**53 -**	8 -	2 +	**50 -**	15 +	15 -
Naked soil				1 +		4 +			**44 -**	1 +		
Construction	**45 +**	16 +	4 -	26 +	6 -	**45 +**	**31 +**	7 +	10 +	**47 +**	14 +	7 -
% Variance	**50**	23	15	**53**	27	10	**56**	**26**	**11**	**49**	25	14

2.1. Historical analysis of supercolonies at first detection locations

We wanted to know whether the Main supercolony could have benefited from an historical advantage over the Catalonian and Corsican supercolonies, due to its first introduction. Thus, we examined which supercolony is present in the locations where the Argentine ant was first detected in each island. To know locations and dates of the first detection of the invasion of the Argentine ant in each island we used available information in the literature and our own knowledge. To know which supercolonies could have been introduced at these sites, we used data from our own sampling or from the sampling of our colleagues. We got information for 19 islands including Corsica and all Balearic Islands; we also included some Atlantic Islands such as the Canary Islands, Madeira and Azores as information was available. We examined which supercolonies inhabit in our days the locations (or the nearest locations) where the Argentine ant was first detected in those islands, or the supercolony inhabiting the whole island when the exact location was not provided.

As information for Corsica was complete and extensive, we re-constructed the history of invasion of the Argentine ant in this island since the first detection date.

3. RESULTS

In Ibiza and Formentera, the number of invaded zones tripled in 12 years, while in Corsica it quadrupled in 9 years. We do not have record of the identity of the supercolonies from last survey in Corsica, but in Ibiza and Formentera, the Main supercolony has been the one that expanded the most. This was especially noticeable in Formentera: in 2004 there was only one location invaded and it was with the Catalonian supercolony, while in 2016 the number of beaches with the Main supercolony was higher than the number of beaches with the Catalonian supercolony (Fig. 1). Furthermore, from 12 beaches invaded unsuccessfully in Ibiza and Formentera in 2016, four of them were already invaded in 2004, meaning that in 14 years the Argentine ant has not been able to displace completely the native ant community. Finally, we also observed that none of the invaded locations have become uninvaded through time.

3.1 Factors affecting the invasion (invaded vs. uninvaded)

For Ibiza and Formentera, we obtained three principal components that represented the environmental variables (Table 2a). The first dimension (70 % variance explained) accounted mostly for the amount of dense vegetation (with negative relationship) and, to a lesser extent, it was positively related with the percentage of surface with human construction. Human construction was mostly represented in the second dimension (with negative relationship) and to a lesser extent it was positively related with the amount of scarce vegetation and negatively with dense vegetation. Finally, the third dimension was represented mostly the amount of scarce vegetation (with a positive relationship) and, at in a lesser extent, it was positively related with the surface of human construction and negatively with the surface of beach.

When we used these three environmental components, together with the spatial variables, to model the invasion status of the beaches in Ibiza and Formentera, we found that the unique significant variable was the first dimension of the PCA ($\chi^2 = 4.32$, $p = 0.037$, N = 56, Table 3a). This means that invaded beaches had a lower relative amount of dense vegetation than the uninvaded beaches, but a higher relative amount of human construction surface (Fig. 3a).

In Corsica, we obtained also three principal components that accounted for the environmental variables (Table 2b). The first dimension (50% variance

Table 3. Effect of the environmental and spatial variables on different aspects of the Argentine ant's invasion process in (a) Ibiza and Formentera Islands and (b) Corsica. Environmental variables are represented by the selected dimensions in each PCA (Dim. 1, Dim. 2, Dim. 3) together with the length of the beach (Length), and the minimum distance to the closest invasion beach or building (Close inv. and Close buil. respectively). Analyses of the invasion process represent invasion status (invaded vs. uninvaded), invasion success (successful vs. unsuccessful invasion), differences between supercolonies (main vs. Catalonian or Corsican) and change of status (change from uninvaded to invaded vs. no change). s indicates the sign of the relationship.

(a)	Invasion			Success			Supercolony			Change Status		
IBI-FORM	χ^2	p	s	χ^2	p	s	χ^2	p	s	χ^2	p	s
Dim. 1	**4.32**	**0.037**	**+**	3.50	0.061	+	1.47	0.226	+	3.34	0.068	-
Dim. 2	0.01	0.941	-	1.95	0.163	-	0.65	0.422	-	0.01	0.923	-
Dim. 3	1.38	0.241	-							1.02	0.314	+
Length	0.17	0.680	+	**11.54**	**<0.001**	**-**	0.765	0.382	+	0.29	0.591	-
Close inv.	1.92	0.165	-	1.25	0.264	+	2.479	0.115	+	0.08	0.776	+
Close buil.	0.08	0.774	-	0.02	0.898	-	0.315	0.575	-	0.19	0.661	+

(b)	Invasion			Success			Supercolony			Change Status		
CORSICA	χ^2	p	s	χ^2	p	s	χ^2	p	s	χ^2	p	s
Dim. 1	0.14	0.712	-	0.37	0.544	+	--	--	--	0.82	0.365	-
Dim. 2	0.26	0.613	-	0.25	0.617	+	--	--	--	0.15	0.697	-
Dim. 3	2.38	0.123	+	0.14	0.711	-	--	--	--	0.02	0.900	+
Length	0.05	0.816	-	0.02	0.875	+	--	--	--	0.10	0.755	+
Close inv.	**10.11**	**0.002**	**-**	0.31	0.577	+	--	--	--	**11.95**	**<0.001**	**-**
Close buil.	0.02	0.90	-	0.00	0.957	+	**4.40**	**0.036**	--	0.03	0.868	**-**

explained) represented the relative amount of human construction (with a positive relationship) and the relative amount of scarce vegetation (with a negative relationship). The second dimension accounted mostly for the relative amount of human constructions (positively related). The third dimension accounted mostly for the relative amount of dense vegetation (positively related) and in a lesser extent for the amount of scarce vegetation (negatively related).

When we modeled the invasion status of the beaches in Corsica, we found that the distance to the closest invaded area was the only variable that increased significantly the likeliness of a beach to get invaded ($\chi^2 = 10.11$, $p = 0.002$, $N = 59$, Table 3b). Thus, invaded beaches had significantly less distance to the nearest invaded beach in the previous survey (Fig. 3b).

3.2 Factors affecting the success of invasion

In Ibiza and Formentera, we obtained two principal components that represented the environmental variables (Table 2a). There were quite similar to the first two dimensions of the precedent analysis. The first dimension (63% variance explained) accounted mostly for the relative amount of dense vegetation (with a negative relationship) and, to a lesser extent, it was positively related with the relative amount of human construction. Second component was represented mostly by the relative amount of human construction (negatively related), and in a lesser extent it was positively related with the relative amount of dense vegetation.

When modeling the success of the invasion in Ibiza and Formentera adding these two environmental dimensions and the spatial variables, we found that invasion success was influenced significantly and negatively by only one variable: the length of the beach ($\chi^2 = 11.56$, $p < 0.001$, $N = 56$). In short beaches the likelihood of a successful invasion is greater than in large beaches (Fig. 3c).

In Corsica we found three principal components representing the environmental variables (Table 2b). The first dimension (53% variance explained) was mainly represented by the relative amount of surface with scarce vegetation, with a negative relationship. The relative amount of human constructions and naked soil were represented to a lesser extent, with a positive relationship. The second dimension accounted mostly for the relative amount of

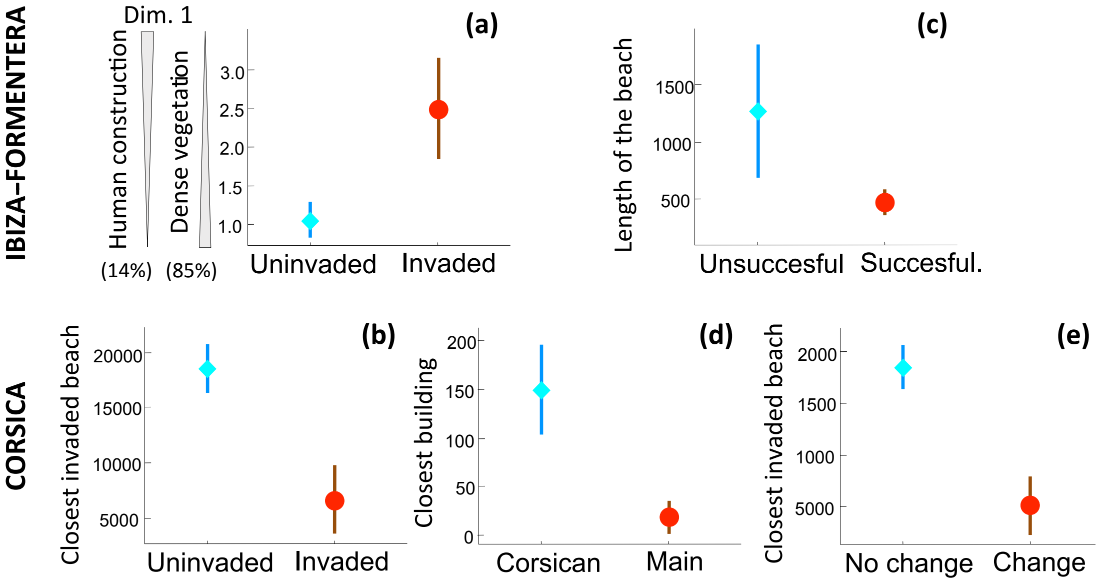

Figure 3. Characteristics of beaches (average ± SE) in relation to Argentine ant invasion. (a) Environmental dimension 1 for invaded (red circles) or uninvaded (light blue diamonds) beaches in Ibiza and Formentera; in the Y axes appear the two variables that explain this dimension, their direction in the dimension, and the percentage of variance explained in the dimension. (b) Minimum distance (m) to the closest invaded beach in the past (9 years before) for invaded (red circles) or uninvaded (light blue diamonds) beaches in Corsica. (c) Length of the beach (m) for successful (red circles) and unsuccessful (red circles) invaded beaches. (d) Minimum distance (m) to the closest building among supercolonies in Corsica. (e) Minimum distance (m) to the closest invaded beach in the past for beaches that remained uninvaded (light blue diamonds) or that changed to invaded (red circles) in the second survey in Corsica.

dense vegetation (positively related), and in a lesser extent with the relative amount of beach (negatively related). The relative amount of human constructions was the main variable represented in the third dimension (and with a positive relationship), but in a lesser extent the relative amount of beach and dense vegetation surface (negatively related) and the relative amount of scarce vegetation (positively related) were also represented (Table 2b).

None of these components or the spatial variables seemed to be relevant for the success of the invasion in Corsica (Table 3b).

3.3 Factors affecting the spread of the three supercolonies

For Ibiza and Formentera, two principal components were selected to represent the environmental variables (Table 2a). The first dimension (63% of the variance explained) was represented by the relative amount of dense vegetation in the beach (negatively related) and to a lesser extent, by the relative amount of human constructions (positively related). The second dimension explained the same two variables, but both with a positive relationship.

Although the Main supercolony has expanded broader in Ibiza and Formentera (from 2 to 5 beaches for the Catalonian supercolony and from 8 to 23 for the Main supercolony, Fig. 1), there were no factors favoring differently their expansion (Table 3a).

For Corsica, there were three the principal components that accounted for the environmental variables (Table 2b). The first dimension (56% variance explained) accounted mostly for the relative amount of scarce vegetation (with a negative relationship) and of human construction (with a positive relationship), and to a lesser extent, with the relative amount of dense vegetation (negatively related). The relative amount of dense vegetation was the main variable explaining the second dimension, with a positive relationship. The third dimension accounted mostly for the relative amount of beach and naked soil, both with negative relationships, and in a lesser extent for the relative amount of human constructions (negatively) related.

As the sampling size for this analysis was low (n = 10), we performed a forward stepwise regression. Distance to building was the only variable showing significant differences between the Main and the Corsican supercolonies (χ^2 = 4.40, p = 0.036, N = 59; Table 3b). The negative sign in this case means that locations with building very close to the shore where more likely to be invaded by the Main supercolony than by the Corsican supercolony (Fig. 3d).

3.4 Factors affecting the change of status from uninvaded to invaded (change vs. no change)

As none of the locations invaded in the past appeared uninvaded when we sampled them posteriorly, the data used here only corresponded to beaches that were uninvaded in the first sampling (Table 1). In Ibiza and Formentera, we obtained three principal components to represent the environmental variables (Table 2a). The first dimension (47% variance explained) represented the relative amount of dense vegetation (with a positive relationship) and to a lesser extent, the relative amount of human construction (with a negatively relationship). The relative amount of human construction was the main variable represented in the second dimension (positively related), and in a lower extent the relative amount of dense and scarce vegetation (positively and negatively related, respectively). Finally, the third dimension was represented mostly by the relative amount of scarce vegetation (with a positive relationship) and, at a lesser extent, it was positively related with the relative amount of human construction and negatively with the relative amount of beach.

When modeling the change of status in Ibiza and Formentera adding these environmental dimensions and the spatial variables, we found that none of the variables seemed to affect to this process (Table 3a).

For Corsica, we obtained also three principal components that accounted for the environmental variables (Table 2b). The first dimension (49% variance explained) accounted mostly for the relative amount of human construction (with a positive relationship) and scarce vegetation (negatively related). The second dimension was explained by the relative amount of beach (negatively related), and to a lesser extent by the relative amount of scarce vegetation and human constructions (positively related). The last dimension was represented mainly by the relative amount of dense vegetation (with a positive relationship), and in a lesser extent, by the relative amount of scarce vegetation (with a negative relationship).

The model with these environmental dimensions and the spatial variables revealed that distance to an invaded beach in the past affected negatively the probability of suffering a change of status ($\chi^2 = 11.95$, $p < 0.001$, N = 59; Table 3B). Uninvaded beaches that were closer to invaded beaches were more likely to be invaded (Fig. 3e).

3.5 Supercolonies at first detection locations

Knowing the locations where the Argentine ant was first detected at each island, we assigned to these locations the supercolonies inhabiting in our days. We could assign a supercolony for most of the studied islands, including all Balearic Islands and Corsica (Table 4). First assessments of supercolonies for each island were made between 2002 for the assessments done in Madeira and Azores, to 2013 in Mallorca. First detection dates of Argentine ant in these islands ranged from before <1858 in Madeira to 2004 in Formentera. Assuming supercolonies do not change temporally in a given location, as we have shown with the data of Ibiza and Formentera, we suggest that the Main supercolony was probably the first introduced in all these groups of islands, except in Formentera (Table 4).

In Corsica the first detected location with Argentine ant was Calvi in 1959 (Bernard 1959), and the supercolony that was present in Calvi in 2007-2008 was the main supercolony. The first location with the Corsican supercolony (assessed in 2007-2008) could correspond to Propriano, when the Argentine ant was first detected in 1976 (Casevitz-Weulersse 1989). Since 1959 to the last sampling in 2007-2008 in Corsica (48 years), the Corsican supercolony seems to have expanded to more locations than the Main supercolony (Fig. 4).

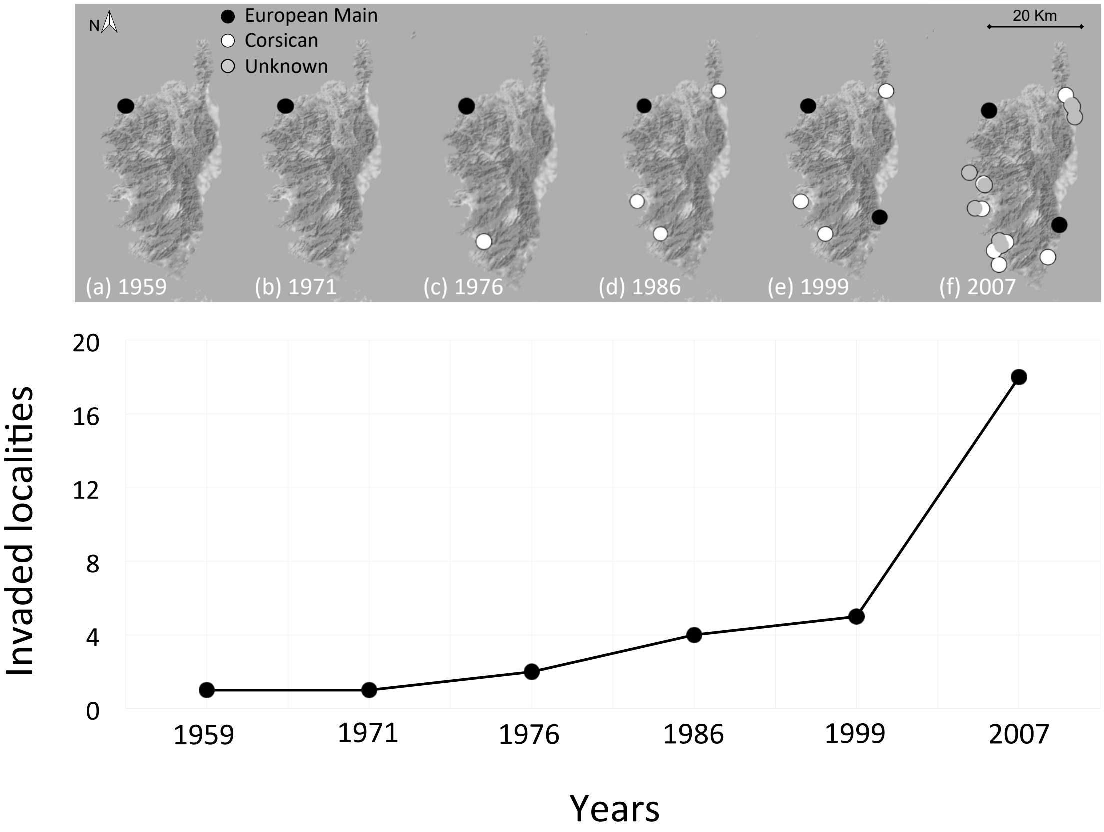

Figure 4. Expansion of the Argentine ant in Corsica since its first detection, and supercolonies that are present in our days: (a) 1959, (b) 1971, (c) 1976, (d) 1986, (e) 1999, and (f) 2007-2008. Data for (a) come from Bernard (1959); data for (b, c and d) come from Casevitz-Weulersse (1989); data for (e) come from Casevitz-Weulersse et al. (1999); and data for (f) come from Blight et al. (2009).

Table 4: First detection dates (FDD) for the Argentine ant, locations, and supercolonies that have been observed in the nearest locations, for some islands archipelagos in South Western Europe. Supercolonies abbreviations are M for the Main, Ca for the Catalonian, and Co for the Corsican supercolonies. The nearest supercolony to the earliest record is in the column "Near", while other supercolonies of each island are in the column "Other" with the number of locations in brackets (N).

Archipelago Island	Location	FDD	Ref	Supercolonies		Supercolony assessment	
				Near	Other (N)	Date	Author
Balearic Islands							
Mallorca	Soller Port	1956	2	M	M (44), Ca (4)	08 / 2013	AT (XE)
Menorca	Maó Port	1976	4	M	M (14)	10/ 2004	KG
Ibiza	Ibiza city	1976	5	M	M (8), Ca (3) M (20), Ca (3)	09 / 2004 09 / 2016	KG & XE SCC
Formentera	La Savina Port	2004	9	Ca	Ca (1) M (3), Ca (2)	09 / 2004 09/2016	KG & XE SCC
Corsica	Calvi	1959	2	M	M (2), Co (8)	2007-8	OB
Canary Islands		1909	15				
El Hierro	Valverde	1950	12	M	M (2)	12 / 2003	7
Lanzarote	Ye	1988	8	M	M (3)	08 / 2005	VB
Fuerteventura	Betancuria	1992	8				
Gomera	--	1927	15	M	M (2)	08 / 2004	XE
Teneriffe	La Orotava	1909	10	M	M (1)	04 / 2006	XE
Gran Canaria	Multiple[a]	1927	15	M	M (2)	12 / 2005	XE
Palma	Los Llanos, San Andrés	1927	15	M	M (2)	04 / 2006	XE
Madeira							
Madeira	Funchal	<1858	12	M	M (6)	2002	14
Porto Santo	Multiple[b]	2002	13	M	M (1)	2002	14
Deserta Grande		2003	13				
Azores							
no locality		<1921	3				
Terceira	Angra de Heroismo	1930	6	M	M (2)	2002	14
Sao Miguel	Punta Delgada, Furnas	1930	6	M	M (3)	2002	14
Flores	Calheta, Ribeira do Salto	1938	11				
Sao Jorge	--	1929	6				
Pico	Baıá Cachorro	1987	1				
Santa Maria	Fonte do Mourato	1957	16				

Ref: refences for the earliest records in each island: [1]Ashmole et al. (1996), [2]Bernard (1956), [3]Chopard (1921), [4]Comín (1977), [5]Comín & Espadaler (1984), [6]Donisthorpe (1936), [7]Espadaler (2007), [8]Espadaler & Bernal (2003), [9]Gómez & Espadaler (2006), [10]Stitz (1917), [11]Wellenius (1949), [12]Wellenius (1955), [13]Wetterer et al (2007), [14]Wetterer & Wetterer (2006), [15]Wheeler (1927), [16]Yarrow (1967).

DA, AA: date of assessment of supercolony identity; and authors or publications of the assessment: AT: A. Tucci; KG: K. Gómez; SCC: S. Castro-Cobo; VB: V. Bernal; XE: X. Espadaler.

Multiple locations: [a]Puerto de la Luz, Las Palmas, Telde, Teror, San Lorenzo, Arucas, Galdar; [b]Airport, Cabeça da Ponta, Camacha, Campo de Baixo, Pico do Castelo, Ponto, Ponta da Conveira, Serra de Dentro, Serra de Fora, Vereda da Terra, Vila Baleira.

4. DISCUSSION

We followed the invasion of the three Argentine ant European supercolonies in three Mediterranean islands for 9 years, in the case of Corsica, and 12 years in the case of Ibiza and Formentera. Our study showed how the Argentine ant is constantly expanding in the three islands, especially the Main supercolony that expanded more than the Catalonian in Ibiza and Formentera (82%), but less than the Corsican in Corsica (20%). We observed that all beaches that were invaded in the first sampling remained invaded. Moreover, more than half of the beaches that were invaded unsuccessfully in the first sampling still remained unsuccessfully invaded 12 years later, meaning that the Argentine ant invasion is not always successful, even many years after invasion. Environmental and spatial variables helped us to corroborate the importance of human presence for the invasion of the Argentine ant: the likeliness of a beach to be invaded in Ibiza and Formentera was negatively related with the amount of dense vegetation but positively related with the amount of human constructions in the area. Moreover, the Main supercolony was found more likely invading beaches when there was a building close by than the Corsican supercolony. Finally, in Corsica, beaches that were invaded or got invaded during our study were the ones closer to invaded beaches, what suggest the importance of secondary introductions in the expansion of the Argentine ant.

4.1 Human-linked dispersion patterns

Biological invasion in islands are very linked to human dispersal. Several studies have proven how humans have been introducing species through commerce or tourism over centuries (e.g. Courchamp et al. 2017). This is especially the case of invertebrates, such as beetles or ants (Marini et al. 2011; Bertelsmeier et al. 2018). Remote and inhabited islands with presence of docks have higher rates of exotic species because they facilitate boat traffic and therefore, the entrance of new species (Rizali et al. 2010; Inoue et al. 2013; Moriguchi et al. 2015). We have not found association between the presence of docks at the beaches and Argentine ant invasion. The most likely explanation is that our study islands have some main ports, which are the ones that more likely have the main commercial and touristic maritime traffic. Then, small docks at beaches, even if used by tourists using boats, seems to be unrelated with the

Argentine ant invasion in the studied islands.

Secondary introductions of invasive ants from the main port entries have been showed to be very frequent in another countries, such as the USA and New Zealand (Berstelmeier et al. 2018). Following these authors, the percentage of secondary introductions for the Argentine ant in these countries was almost 100%. Our results showed that this seems to be the case also in Corsica, the largest of the three studied islands where both, existing invaded beaches and newly invaded beaches, were the ones that were closer to already invaded ones. The history of the Argentine ant invasion in Japan also showed that secondary introductions by human-mediated jump dispersals linked locations with similar supercolonies (Inoue et al. 2013). In Japan the cause of some of these jumps was permanent ship routes among ports or commercial exchanges between locations (Inoue et al. 2013).

The presence of human settlements, as the presence of irrigated parks and gardens nearby, are suitable habitats for the Argentine ant (Heterick et al. 2000; Gomez & Espadaler 2006). In Ibiza and Formentera, invaded beaches seemed to have less amount of relative dense vegetation but higher amount of human constructions than uninvaded beaches. Argentine ant can spread to natural areas (Holway et al. 2002; Menke & Holway 2006; Angulo et al. 2011), but its success is closely related to the distance to the nearest urban area (Moriguchi et al. 2015; Vonshak & Gordon 2015).

4.2 Argentine ant invasion invasions: not always successful

Although the Argentine ant is considered a very successful invader, our survey showed that, interestingly, its invasion it is not always as successful as expected. In almost half of the invaded beaches in Ibiza and Formentera, and in one third in Corsica, the Argentine ant co-occurred with native species (Table1). Beaches that were unsuccessfully invaded were only characterized by a longer shoreline than beaches successfully invaded, while distance to the closest invaded beach was similar between beaches successfully or unsuccessfully invaded. Moreover, during 12 years, the Argentine ant has been co-occurring with the native community in four beaches (of the 12 that were initially invaded unsuccessfully), without been able to displace native species as it usually does when it invades successfully (Holway et al. 2002). This lack of success could be produced by the lack of suitable abiotic conditions, such as high/low temperature or moist (Holway et al. 2002; Menke & Holway 2006). However, although we don't have specific measures of temperature and moisture for these beaches, we know that first, the shorelines of the islands seem to be very suitable habitats for the Argentine ant (Giraud et al. 2002; Gomez & Espadaler 2004); second, these beaches with unsuccessful invasions are interspersed among beaches with successful invasions (See Fig. 1); and third, distance to successfully invaded beaches is not higher than distance among other invaded beaches. We thus suggest that abiotic factors, such as temperature or humidity, are not the cause of the prolonged situation of coexistence of the Argentine ant with native species in these locations, although data is needed in order to corroborate this suggestion.

Biotic resistance could be another factor explaining the duration of unsuccessful invasions: richness of native ant community (Marraffini & Geller 2015; Howeth 2017) or the presence of certain species (Hobbs & Huenneke 1992; Wardle 2001; Kennedy et al. 2002; Henriksson et al. 2015) could prevent the success of the invasion. However, this hypothesis should need further sampling. The lack of success in the invasion process might be given not by the number of the species in the area but by the presence of species with certain traits. It has been proved how native species can slow down the spread of the Argentine ant, such as species from the *Tapinoma nigerrimum* group in Corsica (Blight et al. 2010a), and that traits like behavioral dominance or mass recruitment can be important in biotic resistance through competition for resources (Chapter IV). Several dominant species that mass recruit have been able to resist the Argentine ant such as *Crematogaster scutellaeris*, *Lasius grandis, Pheidole megachepala, P. pallidula, Tetramorium forte or Tapinoma sp* (Way et al. 1997; Paiva et al. 1998; Walters & Mackay 2005; Wetterer et al. 2006; Mothapo & Wossler 2014; Blight et al. 2010). More exhaustive studies are needed to know whether some kind of biotic resistance could explain the long-term coexistence between the Argentine ant and native ants in these islands.

Our results show that the Argentine ant remained in all the invaded beaches along the sampling period (12 years), even in locations that were unsuccessfully invaded. Although natural Argentine ant collapses have been observed in some other regions, like in New Zealand, California and Madeira (Lester & Gruver 2016), as it has occurred with other invasive ants (e.g. is *Anoplolepis gracilipes* in

Australia, Cooling & Hoffmann 2015; or *Lasius neglectus* in several European countries, Tartally et al. 2016), Argentine ant collapses or disappearance from an earlier invaded location were not observed in our study sites.

4.3 Differential expansion of supercolonies

Although we did not expect to find differences between the supercolonies in terms of environmental factors that affected its presence, we found that in Corsica the Main supercolony was linked to the beaches with closer buildings to the shoreline. However, sample size was small to infer a general pattern. Similar to other invaded areas maintaining several different supercolonies, we observed that supercolonies in the three islands seem to be excluding each other (Thomas et al. 2006; Sunamura et al. 2009). It has been seen that ants from the Corsican and the Main supercolonies can tolerate each other in a border zone, with no aggression between them (Berville et al. 2013). This may be given by differences in personality traits that are correlated to ecological success of different supercolonies (Blight et al. 2017).

The higher expansion of the Main supercolony is not well explained by the history of invasion in our case. In Corsica, the Corsican supercolony expanded more than the Main supercolony since the introduction of the Argentine ant in Calvi in 1959 (Bernard 1959). This occurred in spite that in Calvi the Main supercolony occurs, suggesting that it could be the first introduced supercolony in the island. Conversely, the Main supercolony expanded more than the Catalonian supercolony in the two Balearic Islands studied. In Ibiza Island, the first detection was in Ibiza city (Comín & Espadaler 1984), where the Main supercolony is located now. However, the Catalonian supercolony was the first detected in Formentera Island in 2004 in La Savina (Gomez & Espadaler 2006). Although this location remains with the Catalonian supercolony, the Main supercolony has occupied four more locations in Formentera while the Catalonian supercolony has not expanded in this island. Thus, except Formentera, the Main supercolony seemed to be the first introduced in the rest of Mediterranean and Atlantic Islands examined. With the exception of Corsica, the higher expansion of the Main supercolony could respond to the invasion history of first and secondary introductions.

Supercolonies are a subject that arises a lot of interest in evolutionary ecology that are still unsolved, because of their origin and their incompatibility with kin selection theory (Tsutsui et al. 2000; Helanterä et al. 2009). Track of the relationships between supercolonies through time, as our study, can contribute meaningfully to the understanding of this paradox. Argentine ant's expansion is a fact, even more considering that other factors of global change can speed up the process, like climatic change, habitat loss or globalization (Bertelsmeier et al. 2015, 2017, 2018). As we showed here, Argentine ant's spread is influenced by the presence of human activity, directly offering a suitable habitat or indirectly, through jump-events. We claim that long-term monitoring is needed together with broad networks of researchers working with this global invader in order to obtain general patterns for the invasion of the Argentine ant supercolonies at the global scale.

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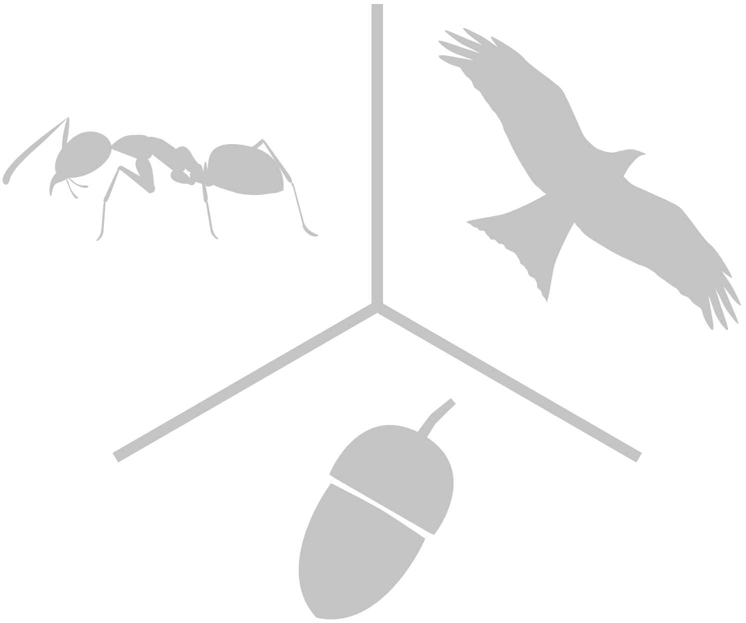
SUPPLEMENTARY MATERIAL

Table S1. Description of the variable ‘Distance to the closest invaded beach’. For those analysis that we knew the identity of the supercolonies, we measured the distance to the nearest beach that was invaded in the past with the same supercolony, showed in the table with an ‘X’. For those analysis that we could not extract that information, we measured the distance to the closest invaded beach in the past, without considering supercolonies, showed in the table with a ‘0’.

	Ibiza/Formentera	Corsica
Invasion	X	0
Success	X	0
Supercolony	X	X
Change Status	X	0



HUMANS AND SCAVENGING RAPTORS FACILITATE ARGENTINE ANT INVASION IN DOÑANA NATIONAL PARK: NO COUNTER-EFFECT OF BIOTIC RESISTANCE

Humans and scavenging raptors facilitate Argentine ant invasion in Doñana National Park: no counter-effect of biotic resistance

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ABSTRACT

Biotic resistance by native communities could have a role in the spread of invasive species. This seems to be the case in the invasion of the Argentine ant, *Linepithema humile,* but only when the environment is unfavorable for the survival of the invader. We studied the progress of Argentine ant invasion through favorable and unfavorable habitats of Doñana National Park across three temporal snapshots covering three decades (1992, 2000, 2016). We assessed biotic resistance of the native community using species richness, as well as dominance and community structure. We also explored the role of abiotic factors (quality of surrounding habitat and spatial variables) and of potential vectors of Argentine ant dispersal across unfavorable areas. We found no evidence of biotic resistance after examining native ant species richness, proportion of native dominant ants, or community structure. On the contrary, invasion proceeded from trees with higher ant species richness, probably because those trees are larger and provide more resources and better protection from aridity. Furthermore, we found evidence that the invasion of new trees across a matrix of unfavorable habitat could be influenced not only by humans, but also by scavenging avian predators, which could act as vectors of ant dispersal through transport of carrion also exploited by the ants. Such leapfrog expansion through mobile predators could represent an overlooked mechanism that would enrich our understanding of invasion dynamics and provide potential opportunities for management of invasive species.

KEY WORDS

Biotic resistance; dominance hierarchies; community structure; dispersal by mobile predators; *Linepithema humile*; raptors

1. INTRODUCTION

Biological invasions are a well-known worldwide threat to biodiversity, reducing or even replacing native species (Sax & Gaines 2003; Simberloff et al. 2013; Kumschick et al. 2015; Li et al. 2016). Different hypotheses can explain the success, spread, and impact of invaders, like propagule pressure, enemy release, biotic resistance or invasion meltdown hypothesis, among others (Catford et al. 2009; Ricciardi et al. 2013; Heger & Jeschke 2014; Jeschke 2014). Ecosystems can be more resistant to invaders due to either abiotic factors, like environmental tolerance to temperature or moisture (Shea & Chesson 2002; Blackburn et al. 2011), or biotic factors, like food resources or natural predators and competitors (Shea & Chesson 2002; Kumschick et al. 2015). According to biotic resistance theory (Elton 1958), native communities with higher species richness should be less susceptible to invasion by exotic species than ecosystems with fewer component species. In this context, interspecific interactions can act as biotic barriers and prevent a new species from persisting in a highly competitive environment (Holway 1999; Parr 2008; Blight et al. 2014).

Ant communities are good models for testing the relative roles of biotic resistance in the spread of invasive species. Competition in ant communities is important in shaping community structure (Parr 2008; Arnan et al. 2012; Cerdá et al. 2013). Moreover, ants' unique life history traits (e.g. social structure, colony foundation, worker polymorphism) make the study of their movements, invasion dynamics and interactions with the native community interesting (Bertelsmeier et al. 2015, 2017). In addition, invasive ants are among the worst world invaders (Lowe et al. 2000), so understanding the role of biotic resistance is important for future management and prevention of its spread. In 1990, Hölldobler and Wilson defined two main types of competition in ants. Exploitative competition refers to the ability of ants to rapidly detect a food resource and recruit a large amount of individuals to exploit it. Interference competition refers to the ability of ants to dominate a food resource through aggression. On the basis of these interactions, ant species have been classified mainly as dominant or subordinate (Vepsäläinen & Pisarski 1982; Savolainen et al. 1989; Arnan et al. 2012; 2017). Dominant species typically shape the species composition of ant assemblages (Cerdá et al. 2013), while subordinate species avoid contact with other species (Arnan et al. 2011). The latter strategy of avoidance allows subordinate species to coexist

(Calcaterra et al. 2016). As a result, high rates of dominant species are not inversely proportional to species richness (Arnan et al. 2011).

The Argentine ant, *Linepithema humile,* is an example of a highly dominant ant (Human & Gordon 1996). It is also a highly invasive species, native to South America, which has successfully established around the globe (Lowe et al. 2000; Suarez et al. 2001). This ant possesses life history traits that make it an exceptionally good invader (Table 1; Arnan et al. 2012; 2017). It is unicolonial (individuals move freely among physically separate nests without showing any kind of intraspecific aggression; Holway et al. 1998; Giraud et al. 2002); its colonies are polygynous (elevated number of reproductive females; Hölldobler & Wilson 1977; Passera 1994) and polydomous (single colonies are divided in

Table 1. Ant species present in the cork oak trees of the study area in 1992 and their life-history traits. Species are sorted by their percentage occurrence (%) in the sampled trees in 1992 (N = 37 trees). Ant species in 2016 and their percentage of occurrence is also showed (N =19 trees). Four life history traits related to competition in ant communities are given, following Arnan et al. (2012, 2017). Behavioral dominance (Dom): dominant (1) and subordinate (0). Number of queens (Queen): polygyny (1), monogyny (0) or both (0.5). Number of nests (Nest): polydomy (1), monodomy (0) or both (0.5). Foraging strategy (Forage): mass recruitment (1), group recruitment (0.5), individual foraging (0). Diet: liquid food (0), seeds (0.5) or insects (1). The Argentine ant is added as a comparison for these traits.

	%		**Life history traits**				
Species	**1992**	**2016**	**Dom**	**Queen**	**Nest**	**Forage**	**Diet**
Linepithema humile	-	-	1	1	1	1	0, 1
Crematogaster scutellaris	76	84	1	0.5	0.5	1	0, 1
Camponotus lateralis	65	42	0	0	0	0.5	0
Colobopsis truncata	57	84	0	0	0	0	0
Lasius lasioides	49	11	1	0	0	0	0, 1
Camponotus cruentatus	41	0	1	0	0	0.5	0, 1
*Cataglyphis spp.**	24	0	0	0	0	0	1
Temnothorax racovitzai	16	0	0	0	0	0.5	0, 1
Tapinoma cf. *nigerrimum*	11	5	1	1	1	1	0
Iberoformica subrufa	5	0	0	0	0	0.5	0, 1
Tetramorium forte	5	0	1	0	0	1	1, 0.5
Cardiocondyla batesii	3	0	0	0	0	0.5	0, 1
Plagiolepis schmitzii	3	0	0	1	0.5	0.5	0
Camponotus fallax	0	37	0	0	0	0.5	0
Lasius grandis	0	21	1	0	0	1	0, 1
Formica cunicularia	0	5	0	0	0.5	0.5	0, 1
Tapinoma erraticum	0	5	1	1	1	1	0

* *Cataglyphis spp.* corresponds to two species *C. floricola* and *C. tartessica*, the latter was described in 2014 and formerly was considered the orange form of *C. floricola* (Amor & Ortega, 2014).

multiple physical nests; Pedersen et al. 2006; Heller et al. 2008); and it is highly aggressive toward other ant species through both physical and chemical attacks (Suarez et al. 1999; Welzel et al. 2018). When it colonizes a new area, the Argentine ant usually displaces the native ant community and produces a cascade of negative impacts on other taxa including arthropods, small vertebrates, and plants (Holway et al. 2002; Sanders et al. 2003; Rowles & O'Dowd 2009; Alvarez-Blanco et al. 2017). *L. humile* has an overall generalized diet including insects, carrion or nectar (Holway et al. 2002, Angulo et al. 2011), and it has been shown that carbohydrate-rich diets provided by aphids favor its success (Rowles & Silverman 2009). The only aspect of this species' natural history that may constrain its colonization potential is the lack of winged queens, which can prevent dispersal across patches of unfavorable habitat (Way et al. 1997; Holway 1998).

Previous work has demonstrated that native ant communities do not show biotic resistance against Argentine ant invasion when abiotic conditions are favorable for the Argentine ant (Way et al. 1997; Holway 1998; Menke et al. 2007; Rowles & O'Dowd 2007; Roura-Pascual et al. 2011). On the other hand, some dominant native species are capable of resisting the invader Argentine ant. For example, species like *Lasius grandis*, *Tapinoma nigerrimum* or *Iridomyrmex rufoniger* can repel the invader when its propagule size is not adequate (Way et al. 1997; Walters & Mackay 2005; Wetterer al. 2006; Blight et al. 2010), while some other species like *Crematogaster scutellaris, Pheidole pallidula* and *Tetramorium forte* can prevent its spread in habitats that are suboptimal for the Argentine ant (Way et al. 1997).

The Argentine ant expansion has mainly affected areas with a Mediterranean climate but can also be limited by abiotic factors, especially temperature and moisture (Carpintero & Reyes-López 2008; Roura-Pascual et al. 2009, 2011).

Although localized resistance offered by dominant species could be effective under certain environmental conditions, spread across abiotic obstacles could be favored by accidental facilitation, such as, human-driven jumps. For example, it has been shown that, at the global scale, long-distance Argentine ant dispersal is typically mediated by unintentional human transport (Suarez et al. 2001). Humans can also enable more local, short-distance jumps (Carpintero et al. 2005;

Angulo et al. 2011), which are otherwise unlikely because of the wingless queens.

In this study, we aim to assess the relative roles of different factors affecting the spread of the Argentine ant at a local scale in a Mediterranean ecosystem with favorable habitats (cork oak trees) interspersed within an unfavorable scrubland matrix. We used a temporal approach, comparing snapshot-annual data of ant communities collected through three different decades (in 1992, 2000 and 2016) during the spread of the Argentine ant in the area. First, we tested whether the native species richness or the proportion of dominant ants in uninvaded cork oak trees in 1992 could be limiting the future invasion by Argentine ants. We hypothesized that trees with low ant species richness or low proportion of dominant ants would be invaded first, following the biotic resistance hypothesis. In order to further explore this hypothesis, we analyzed whether the community composition was related to the process of the invasion. We hypothesized that trees that were invaded first would have a different species composition compared to the ones that were not invaded. Alternatively, we explored the possibility that spread to new trees is facilitated by other factors. In the study area, transport of *L. humile* by humans and by scavenger raptors, which may take ant-infested carrion items to their nests, has been qualitatively suggested to influence the invasion of new trees (Carpintero et al. 2005; Angulo et al. 2011). In these cases, we would expect that the Argentine ant spread would be linked to roads and paths, or to the breeding sites of scavenging raptors.

2. MATERIAL AND METHODS

2.1. Study area

Sampling was conducted in Doñana Biological Reserve of Doñana National Park (37°1’N, 6°33’W), situated on the Southwest coast of Spain, in an open Mediterranean scrubland containing scattered pine forests (*Pinus pinea*) and isolated cork oak trees (*Quercus suber*) (Fig. 1). The study area is inhabited by more than 30 ant species, and the Argentine ant is the only invasive species (Carpintero et al. 2003). The first invasion focal point was the building of El Palacio de Doñana, the Reserve’s field-research station with year-round human presence of some 3-50 people, where the Argentine ant was first detected in 1970 (Angulo et al. 2011). Due to its low tolerance of high temperatures and dry

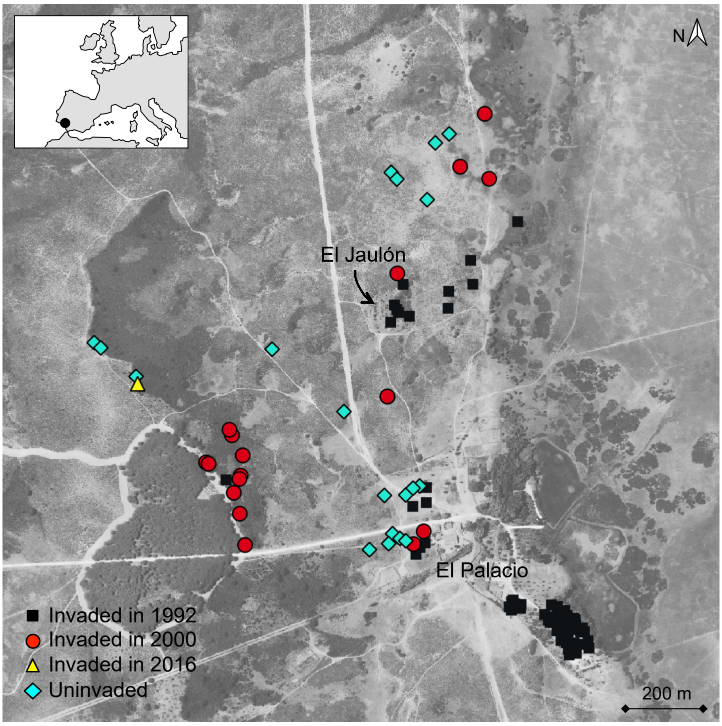

Figure 1. Study area: the main focal points for the invasion of the Argentine ant, El Palacio and El Jaulón, are marked. The study area consists of a matrix of unfavorable dry Mediterranean scrubland containing scattered cork oak trees, which act as favorable habitat patches. Trees that were invaded in 1992 are marked by black squares, trees that were invaded in 2000 are marked by red circles, and trees that were still not invaded in 2000 are marked by light blue diamonds. The only tree invaded between 2000-2016 is marked by a yellow triangle.

habitats, its range in natural habitats is mainly restricted to individual cork oaks or pine forests (Angulo et al. 2011). The first time the Argentine ant was seen in a natural area of Doñana National Park was in the 1980's in a cork oak in El Jaulón (Carpintero et al. 2005), a human construction that very likely constituted a second invasion focal point (Fig. 1). Thus, in our study, isolated cork oak trees constitute our sampling units in order to follow the invasion progress of the Argentine ant.

2.2. Sampling the ant community

In June 2016 we repeated, using the same protocol, the sampling that Carpintero et al. (2005) performed in 1992 and 2000. They sampled the ant community at all the cork oaks within 1.5 km of the invasion focal point, El Palacio. At each tree, during 10 minutes, we looked for the different ant species, native or invasive, patrolling trunk and branches from 0.5 to 2 m high. We considered a tree invaded if the Argentine ant was present on it. In general, when the Argentine ant successfully invades a tree, it replaces all other native ant species, so its detection is very likely (Angulo et al. 2011). Samples of native ant species were collected and kept in 70% alcohol for subsequent identification by the authors in the laboratory.

From the 182 trees Carpintero et al. (2005) sampled, we excluded the ones that were not alive in 2016, and three trees that had two trunks one beside the other were considered only one tree. Thus, we get an initial sample size of 105 trees in 1992, of which only 38 were uninvaded at that time. Of these 38 trees, 18 had been invaded by 2000 and only a single additional tree had been invaded by 2016 (Fig. 1). Therefore, because expansion virtually halted after 2000 in the sampled area, here we focus on the process of invasion that occurred between 1992-2000, which covers a significant invasion expansion. Trees were categorized by their invasion status as “uninvaded” if they remained uninvaded in 2000 (N = 19) or “invaded” if they were invaded in 2000 (N = 18; Fig. 1), with a total number of 37 cork oaks.

2.3. Ant community structure

A matrix of presence-absence of each native ant species found in the 1992 survey was constructed for the 37 trees selected for the analysis. We compared the native ant community between trees that were subsequently invaded in 2000 or remained uninvaded by this year (invasion status). We performed a permutational multivariate analysis of variance (PERMANOVA) using the *adonis* function (package Vegan, Oksanen et al. 2016) in R (R Studio version 1.0.136, RStudio Team 2016).

To graphically represent differences in community structure by invasion status, we performed a non-metric multidimensional scaling test (NMDS), using the *metaMSD* function with 1000 iterations (package Vegan, Oksanen et al. 2016) in R (RStudio Team 2016). This analysis represents the original position of each community in multidimensional space. We chose the appropriate number of dimensions by examining the stress value (correct below 0.1). Trees were differentiated by their invasion status using the *ggplot* function (package ggplot2, Wickham 2009), which draws 95% confidence interval ellipses.

We were interested in whether the ant community of uninvaded trees had changed. We performed a second PERMANOVA and NMDS (as described before) to compare ant communities in 1992 and in 2016, using only trees that remained uninvaded in 2016 (N=19).

2.4. Anthropogenic, biotic and abiotic variables affecting the invasion of new trees

In order to know which parameters affected the invasion of new trees since 1992, we performed a generalized lineal model using the *glm* function (package stats, R Core Team 2015). The dependent variable was the status of invasion (invaded or uninvaded in 2000) and we modeled it with a quasibinomial link distribution. We estimated the following parameters that we fitted to the model as independent variables:

(a) Ant species richness: number of native ant species for each cork oak tree in 1992. Species richness was added to the model in order to test the biotic resistance hypothesis, under the prediction that less species-rich trees would be liable to invasion.

(b) Behavioral dominance of native species: we separated the native species found in 1992 in each cork oak tree by their position in the behavioral dominance hierarchy, classing each as dominant or subordinate, following Arnan et al. (2012, 2017) (Table 1). We estimated the percentage of dominant species from the total number of native species present. Our prediction was that a lower proportion of dominant species would increase the likelihood of invasion.

(c) Spatial variables: The geographic coordinates (latitude and longitude) of each tree were added to the model to take into account the spatial arrangement of trees. We used a geographic information system (QGIS, version 2.18.14, QGIS 2009) to calculate distances from each sampled tree to three potential invasion sources: El Palacio, El Jaulón, and the nearest invaded cork oak, with the tool “measure line”. We only used the minimum of these distances, to take into account the proximity of each tree to the closest potential invasion source.

(d) Surrounding habitat quality: Argentine ant expansion is limited by dryness and high temperatures of the scrubland matrix around cork oak trees (Angulo et al. 2011). The normalized difference vegetation index (NDVI) measures, through satellite imagery, visible and near infrared light reflected by plants, and is an estimate of the density and quality of the vegetation of a certain area (Karkauskaite et al. 2017). NDVI was measured in a circle of 100 m of radius around the trunk of the tree by accessing Landsat images of 30x30 pixels, using the function *create buffer* in QGIS 2.18.14 (QGIS 2009). For each tree we calculated yearly measures as the average of monthly measures (0-3 measures per month were available) and then used the average of the yearly measures from 1992 to 2000. Higher NDVI means more productivity and more vegetation cover, implying lower aridity and thus a better habitat quality for the Argentine ant.

We also measured the perimeter of each trunk (cm) at 1.5 m height, because bigger trees provide more vegetation cover, humidity and lower temperatures than smaller trees (Angulo et al. 2011).

(e) Dispersal by humans: we calculated the minimum distance from each sampled tree to the nearest path. Because humans may inadvertently transport ant queens in their cars or with their belongings, lower distances to paths would mean higher probability of ant queen transport to cork oaks. We used the same tool as in (c) to calculate the minimum distance.

(f) Dispersal by raptors: Black and Red kites (*Milvus migrans* and *M. milvus*, respectively) breed in large numbers in the study area, mainly using cork oaks and pine trees as support for their nest (Sergio et al. 2005). Both species are wide-ranging facultative scavengers, very adept at finding animal carcasses, even very small ones, such as meat items from human refuse (e.g. Kumar et al. 2018). During the breeding season, prey items are usually transported to the nest or to feeding perches, typically located within 10-50 m from the nest. We used historical data from kite surveys conducted between 1992 and 2000 (details in Sergio et al. 2005) to obtain: (1) the total number of kite nests situated in a 50 m radius centered on the trunk of each cork oak, and (2) the total number of nestlings that were raised in those nests each year. Because both variables were correlated ($R^2 = 0.88$, $p < 0.001$, $N = 37$), we chose to include in the model only the number of nests. We assumed that more nests would lead to higher probabilities of accidentally transporting queen ants that were feeding on the carrion item when this was picked up and taken to the nest area by a kite, thus dispersing the ants.

Statistical differences for each of the above independent variables were obtained with the function *drop1* and the F statistic (package stats, R Core Team 2015). We used a backward stepwise procedure in order to obtain a final model that only included statistically significant variables (Table 2).

3. RESULTS

Of the total 105 cork oaks that survived the three decades of study, 67 were already invaded in 1992, 18 were invaded from 1992 to 2000 and just one more had been invaded by 2016 (Fig 1). We also observed trees that were invaded but were found devoid of Argentine ants in subsequent years. This occurred in eight trees: six were invaded in 1992, of which three had native ants in 2000 and the other three had native ants in 2016; two trees that were invaded in 2000 had native ants in 2016.

Table 2. Effects of anthropogenic, biotic and abiotic variables in the invasion of new trees. Statistics for the complete (initial) model and the final model, obtained by a backward stepwise procedure, are provided (N= 37). For the final model the sign of the relationship has been added.

	Complete model		**Final model**		
	F	**p**	**F**	**p**	**Sign**
Species richness	2.53	0.124	17.32	< 0.001	(+)
Dominant species (%)	0.54	0.741	-	-	-
Distance to an invasion source	18.43	< 0.001	56.73	< 0.001	(-)
NDVI	0.03	0.869	-	-	-
Tree perimeter	2.10	0.159	-	-	-
Distance to a path	20.52	< 0.001	34.84	< 0.001	(-)
Number of nests	3.01	0.094	6.68	0.015	(+)
Latitude	0.62	0.440	26.12	< 0.001	(+)
Longitude	1.29	0.266	39.10	< 0.001	(+)

3.1 Differences in ant community structure between invaded and uninvaded trees.

The community analysis did not show significant differences in the native ant community (PERMANOVA, F = 1.63, p = 0.141, N = 37) between the trees that were invaded or uninvaded in 2000. We ran NMDS with two dimensions and had a fair stress value of 0.05. As shown in Fig. 2a, the ant community structure of invaded and uninvaded trees was very similar, the only difference being a wider ant community in uninvaded trees, suggesting that ant communities of the invaded trees could be a subgroup of the ant communities of uninvaded trees.

The ant community of uninvaded trees significantly changed between 1992 and 2016 (PERMANOVA, F = 6.23 p = 0.001, N = 19, Fig. 2b). While the ant community in 1992 contains more species than in 2016, the two communities are largely overlapping, differing in some species that have been substituted for others with similar ecological functions (Table 1).

3.2 Anthropogenic, biotic and abiotic variables affecting the invasion of new trees.

When testing the biotic resistance hypothesis, we found that species richness was selected as an important variable in the final regression model (F = 17.32, p < 0.001, N = 37, Fig 3a, Table 2). However, the sign of the relationship implied no biotic resistance to invasion by the native community. Indeed, we found the opposite result: trees with higher native ant richness were more likely to be invaded. The behavioral dominance hypothesis was also not supported: the percentage of dominant species in each cork oak was not selected as a significant variable in the final model.

Geographic effects were important for the invasion process: the invasion of new trees depended on the distance to the nearest source of invasion (F = 56.73, p < 0.001, N = 37, Fig 3b, Table 2). Latitude and longitude also had a significant effect, showing an invasion pattern that moved preferentially to the north and west (F = 39.10 and F = 26.12 respectively; p < 0.001, N = 37 in both cases, Table 2).

In relation to the tree quality and its environment, neither the perimeter of tree trunk in 1992 nor the NDVI index were selected as important variables in the final

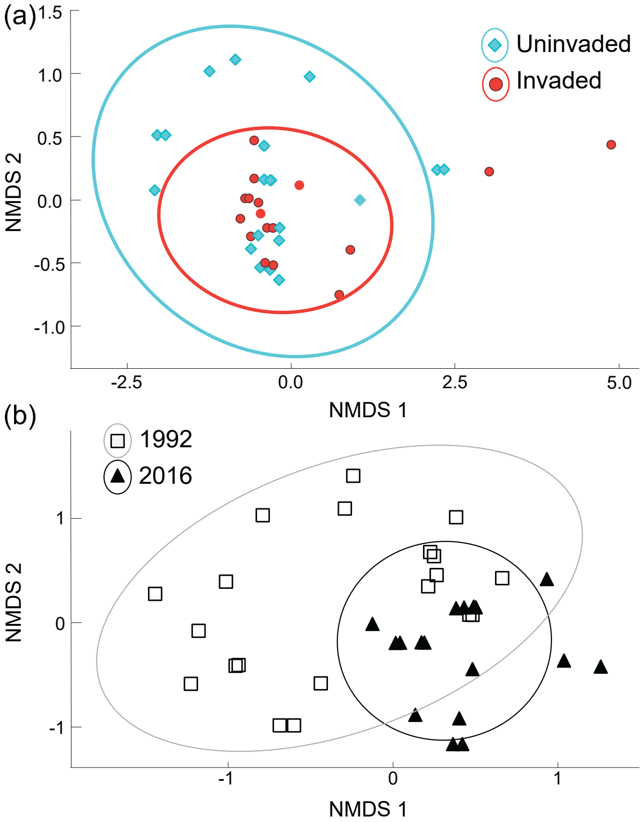

Figure 2. Ordination of ant community similarity (NMDS) for (a) trees that were invaded by Argentine ants (red circles) or that were still devoid of the invading species by 2000 (light blue diamonds); (b) ant communities in uninvaded trees in 1992 (white squares) and in 2016 (black triangles). The ellipses represent the 95% confidence interval for each group.

model (Fig. ESM_1, Table 2). Finally, anthropogenic and raptor facilitation were supported by our results: the likelihood of invasion increased with the proximity to a path (F = 34.84, $p < 0.001$, N = 37; Fig 3c, Table 2) and with the number of kite nests in the immediate surroundings (F = 6.68, p = 0.015, N = 37, Fig 3d, Table 2).

4. DISCUSSION

We followed the invasion of *L. humile* at a local scale during three different decades (1992, 2000 and 2016), studying different biotic and abiotic factors that can influence the process, in a protected area with favorable habitats interspersed with unfavorable ones. We found that the invasion moved from main focal invasion points towards the closest trees and that the likelihood of invasion increased with the proximity to a path. The native ant community did not offer resistance to the invasion, neither in terms of species richness, nor in proportion of dominant species or community structure. However, our results suggest that raptors facilitate ant dispersal towards areas that would not be accessible to the Argentine ant by itself.

Most of the literature dealing with biotic resistance against the Argentine ant shows that native ants are not able to resist the invasion when abiotic conditions are adequate for the invader. Exceptions found in the bibliography include communities that contain dominant native species, such as *Tapinoma nigerrimum* in Corsica, which can limit the spread and establishment of this invader (Blight et al. 2010). Although *T.* cf. *nigerrimum* was found in 11% of the cork oak trees we studied, its presence did not seem to prevent invasion. *T. simrothi*, another dominant species that is ecologically similar to *T.* cf. *nigerrimum,* was shown by

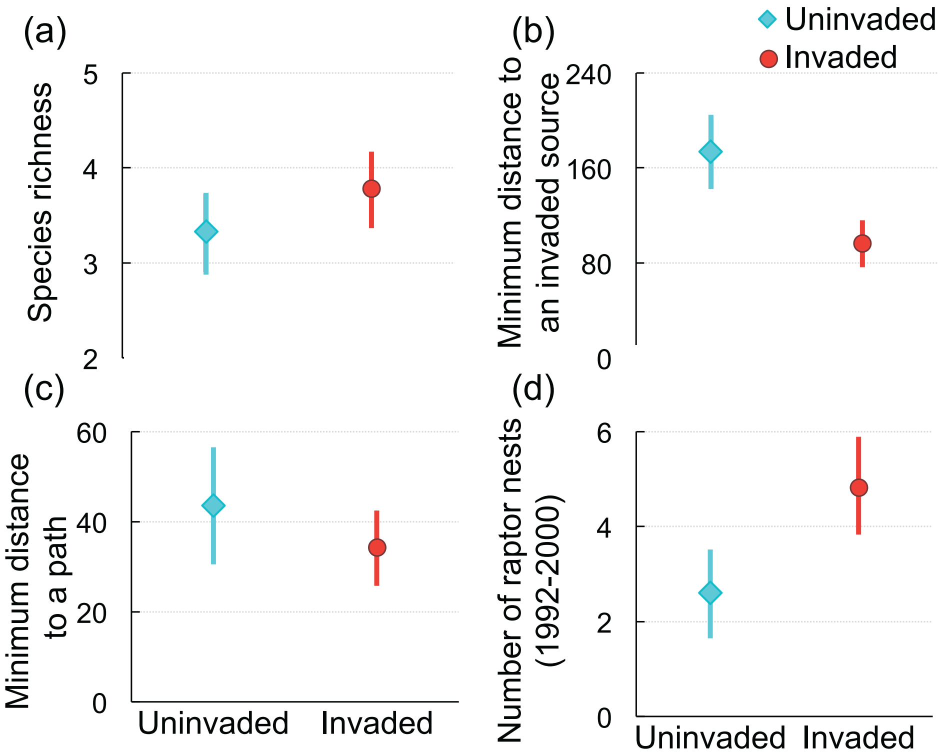

Figure 3. Characteristics of trees invaded (red circles) or uninvaded (light blue diamonds) by the Argentine ant. (a) Average (± SE) of native ant species richness in 1992; (b) Average (± SE) of the minimum distance to an invaded source (invaded tree or invasion focal point); (c) Average (± SE) of the minimum distance to a path; (d) Average (± SE) of total number of nests of scavenger raptors in invaded trees or in trees that remained uninvaded by 2000. Raptor nests include those of *Milvus milvus* and *M. migrans* in a 50 m circle around the tree for the period 1992-2000.

Way et al. (1997) to be displaced by the Argentine ant as well. Dominant species also have a role in the first steps of invasion: a higher propagule size (e.g. a larger Argentine ant colony size) is needed for successful invasion in the presence of dominant species such as *Iridomyrmex rufoniger* in Australia (Walters & Mackay 2005), *Pheidole megacephala* in South Africa (Mothapo & Wossler, 2014) or *Monomorium antarcticum* in New Zealand (Sagata & Lester 2009).

Given the local juxtaposition of habitat conditions, the invasion of new trees in our study area could be accomplished by diffusion (e.g. "budding", where a group of workers and queens colonize new areas on foot) or by leapfrog advances, depending on whether trees are separated by favorable or unfavorable habitat (Suarez et al. 2001). Such jumps would consist of a small propagule size, and thus the effects of dominant species could have a role in preventing such new invasions. Among the five dominant species in our study area, the one that was more frequent in the trees before invasion was the native arboreal ant *C. scutellaris* (located in more than 75% of the trees). This species is aggressive and territorial, but does not always display polygyny and polydomy (two characteristics that if present could make their arboreal nests less vulnerable to Argentine ants); that may be the reason why it was systematically displaced during the invasion (Way et al. 1997; Angulo et al. 2011). When abiotic conditions are unfavorable for the invasion, native species can coexist and compete with Argentine ants for resources, thus slowing down the invasion (Menke et al. 2007). Examples include *Solenopsis molesta, Leptothorax andrei* and *Prenolepis imparis* in Southern California (Suarez et al. 2007).

Despite the above, neither the proportion of dominant species nor species richness prevented the spread of the invader in our system. Studies in other Mediterranean climates such as the chaparral of Southern California (Menke et al. 2007) and the coastal scrub of Australia (Rowles & O'Dowd 2007) have also shown a lack of biotic resistance by native ants when abiotic conditions were favorable for the invasion. However, biotic resistance has been reported to be more effective in natural than in urban habitats (Helford et al. 2012). Although Doñana is a natural area, the invasion is for the moment concentrated in the surroundings of a small, permanent human settlement, which could help the Argentine ant to persist against natives.

The analysis of ant community structure suggested that trees that resisted the invasion had a wider community structure than the trees that were invaded. This does not mean that they were simply composed of more species, but rather that they were organized differently, suggesting the possibility that the key to biotic resistance against invasion may not be the number of species or percentage of dominants, but rather a certain combination of species. Moreover, natural ant communities change over time, especially in isolated trees (Gove et al. 2009; Donoso 2017). In our case, ant communities of uninvaded trees differed between 1992 and 2016. The changes did not seem a result of Argentine ant invasion, but rather consisted of substitution between ecologically similar native ant species. More research should be done in order to assess this effect.

However, contrary to the biotic resistance hypothesis, our results showed that cork oaks with higher ant species richness were invaded first. Holway (1998) found that, although invasion rate was independent of native ant richness, it increased with native ant abundance. In fact, larger trees are the ones that have resources capable of maintaining more species (Kurz-Benson et al. 2006). Although in our analyses the perimeter of the tree was not a significant variable for the invasion, we also observed that larger trees had more ant species (see Fig. ESM_2a). Larger trees also support higher abundances of aphids (Ward et al. 1998), which may facilitate invasion because aphid honeydew is one of the main food sources for the Argentine ant (Shik & Silverman 2013). Both the native ant *C. scutellaris*, and the invasive Argentine ant use honeydew resources in cork oaks (Abril et al. 2007). And in our study area, ant abundance in trails on the tree trunk was correlated with the size of the tree (see Fig. ESM_2b), suggesting that bigger trees sustain bigger colonies, probably because of the greatest availability of resources, such as aphids. In addition, larger tree crowns provide more shade, which is likely to result in temperatures conducive to Argentine ant survival. Holway (2005) and Angulo et al. (2011) found that Argentine ants were more abundant in habitats with more vegetation cover and moisture, while in the scrubland they were less abundant and more likely to coexist with native species (Way et al. 1997; Holway 2005; Menke et al. 2007; Rowles & O'Dowd 2007; Roura-Pascual et al. 2011).

The presence of unfavorable dry scrubland areas separating the favorable habitat constituted by the cork oak trees has very likely slowed the expansion of the Argentine ant during these past decades. According to the review of Suarez et al. (2001), the maximum annual rate of spread of the Argentine ant invasion is 154 ± 21 m (range 15 - 275 m), which means that in Doñana it could have spread as far as 3.7 km (range 360 m - 6.6 km) between 1992 and 2016. However, since 1992 only 19 of the 37 uninvaded trees in the study area were colonized, and since 2000 only one new tree was colonized, while 18 trees remain uninvaded. This occurred within a sampling area of 1.5 km radius from the main invasion focal point, where the maximum distance from the main invasion focal point to an invaded tree was 509 m in 1992. Thus, the local Argentine ant population does not seem capable of saturating its already colonized range. Moreover, we detected eight invaded trees that were subsequently devoid of Argentine ants and which were colonized again by native ants (six of these trees were already invaded in 1992 and two were invaded in 2000). This is not a rare situation. Menke et al. (2018) also found evidence of invasion retreat in Northern California, probably caused by the severe drought that the State had suffered during four consecutive years. Cooling et al. (2011) found a population collapse of the Argentine ant in New Zealand that allowed the native populations to recover, perhaps due to abiotic conditions like high rainfall or to inbreeding depression.

Although trees that were closer to the invasion source were invaded earlier, the Argentine ant has reached areas that are surrounded by unfavorable and uninvaded habitats. It is widely known that humans are dispersal vectors of the Argentine ant (Suarez et al. 2001; Ward et al. 2005; Boser et al. 2017), and proximity to paths frequently used by humans was previously suggested as a factor contributing to spread across unfavorable areas in Doñana National Park (Carpintero et al. 2005). Our results add quantitative support to this idea, showing that trees that were closer to a path were invaded earlier.

Moreover, Carpintero et al. (2005) proposed that, among vertebrates, scavenging raptors could facilitate the invasion of new trees across unfavorable habitat, and our results lend quantitative support to this hypothesis. The invasion of a tree was related to the abundance of kite nests in the surrounding habitat. Because the Argentine ant is a scavenger species (Angulo et al. 2011), it is possible to find it feeding on animal carcasses or meat refuse from humans. Queens are also sometimes present on such food items (authors' pers. obs.). When ant-infested food items are picked up and transported by kites to their nest areas, there is a chance that the propagule could be transported to an area inaccessible to the Argentine ants alone. This scenario could explain the leapfrog invasion of some isolated trees in the north of our sampling area (see Fig. 1). Although this mode of spread may be of relatively minor importance compared with the large-scale, sometimes inter-continental, human-mediated jump dispersal, it could be of importance in natural areas where large avian predators are common, where human presence is less pervasive, or where management actions have been attempted to reduce human-mediated ant dispersal. For example, at our study site, measures to prevent the inadvertent transport of Argentine ant propagules by humans have been established since 2004. These measures include substituting concrete for wood in fencing, directives for secure transport of food, garbage and other human materials from houses to the rest of the protected area, and adequate transport and conservation of organic materials used in experiments or for scientific collections. Additional routes of human-related dispersal should be taken in account when studying invasion patterns: soil movements (used for path restoration and other infrastructures), translocations of plant specimens grown in invaded areas, or constructions of underground infrastructures (which ants could use as refugia or dispersion routes to new areas). Transport by raptors cannot be prevented, especially by wide-ranging raptors such as kites that may forage in rubbish dumps tens of kilometers outside of the protected area (Heredia et al. 1991).

Independently of the local situation, the possibility that mobile vertebrate predators, such as avian facultative scavengers, can act as vectors of invasive species is relatively new in invasion ecology and adds a new dimension to invasion management and prevention. Note that some avian facultative scavengers are opportunistic-adaptable species that can be locally common in both urban and natural areas. For example, black kites are extremely abundant within urban environments of Africa and southern Asia, where they subsist almost entirely on human subsidies and refuse, reaching the highest raptor densities ever recorded (Kumar et al. 2018). Their potential as vectors of ant dispersal in these environments, or their synergy with human dispersal, would be even greater than portrayed here. In conclusion, our study lends support to previous ones suggesting that biotic resistance *per se* is unlikely to halt Argentine ant invasion, even in environments with an overall unfavourable matrix. Furthermore, colonization maintenance and spread may be supported not only by humans but also by mobile predators, whose dispersal capabilities should be considered when planning the management of invasive species.

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SUPPLEMENTARY MATERIAL

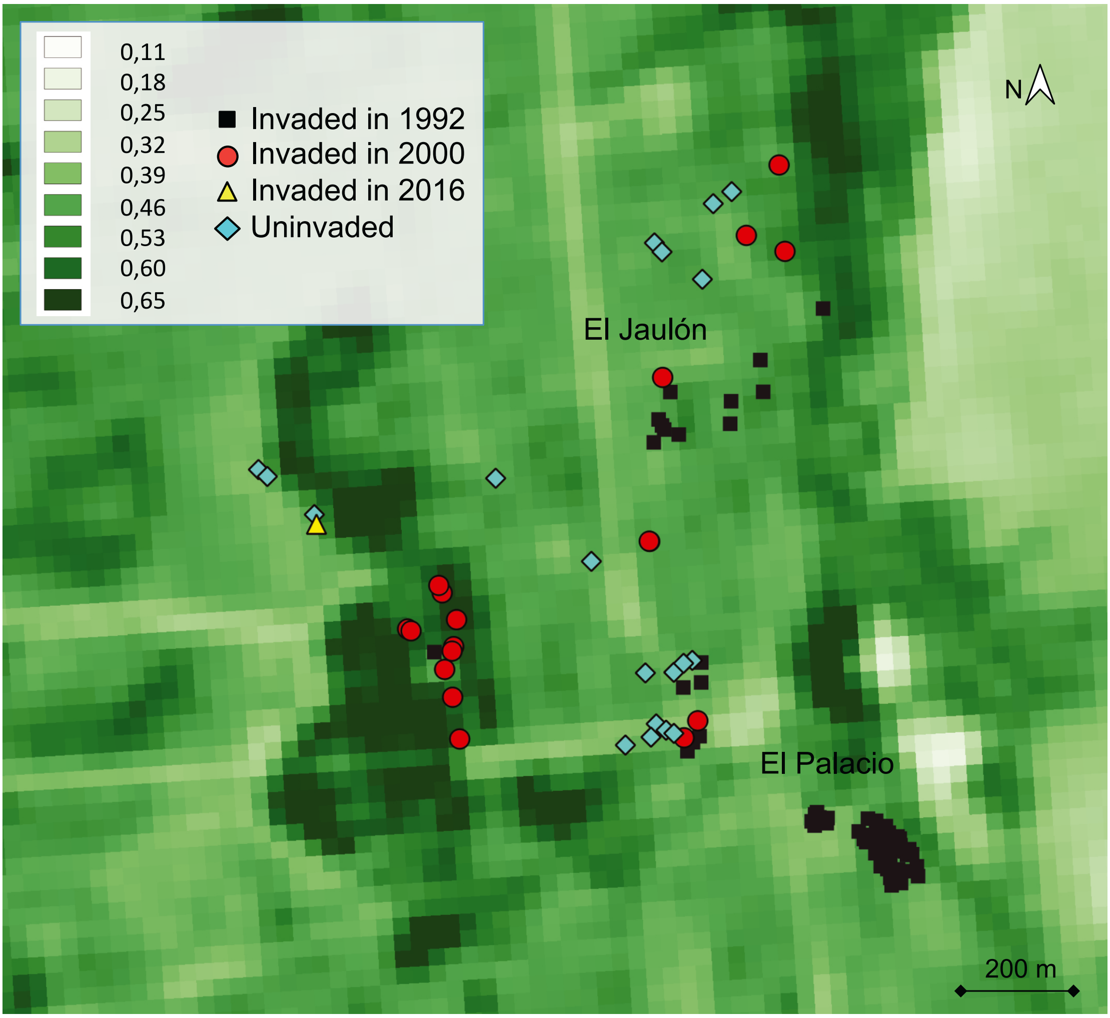


Figure S1. Mean NDVI values (1992-2000) in the study area. Data is provided for 30x30 pixels. Higher values represent higher density and quality of the vegetation (see Methods). In our case higher values corresponded to trees, mainly cork oak trees, pine trees, and poplars, and to the wet scrubland, which is constituted mainly by *Erica* spp., *Ulex* spp. and *Rubus ulmifolius*. The lowest values corresponded to paths, marshlands and ponds. Intermediate values corresponded to the dry scrubland constituted mainly by *Halimium* spp., *Ulex* spp., *Cistus* spp., *Lavandula stoechas* and *Thymus mastichina*.

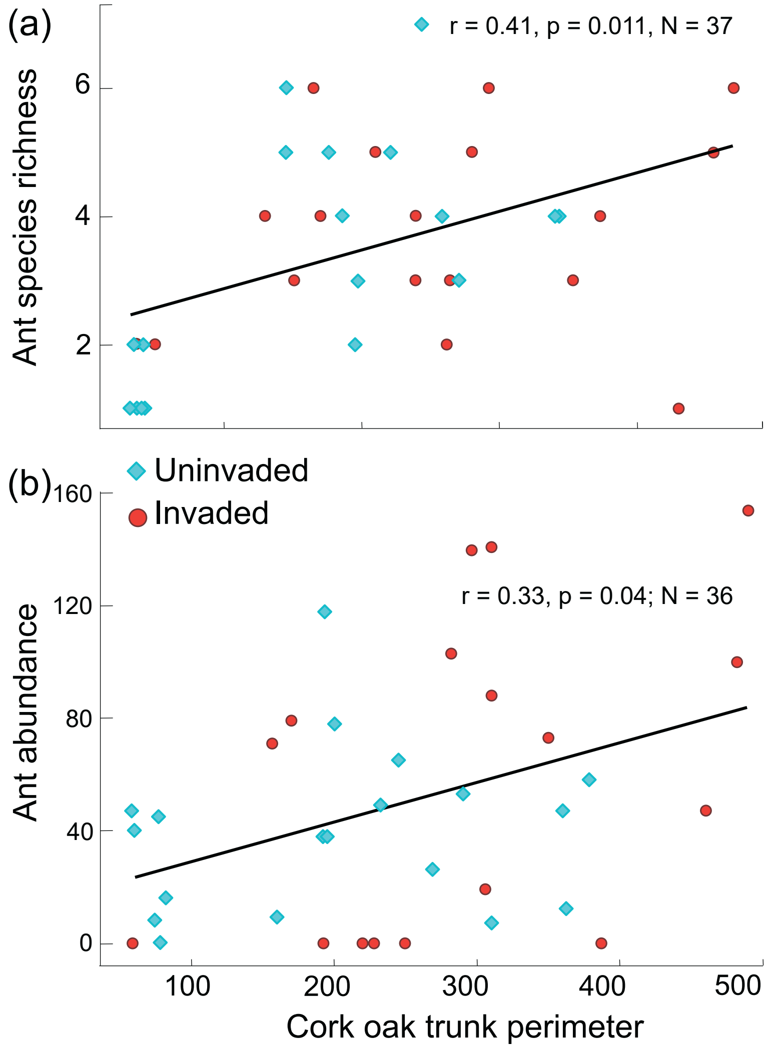
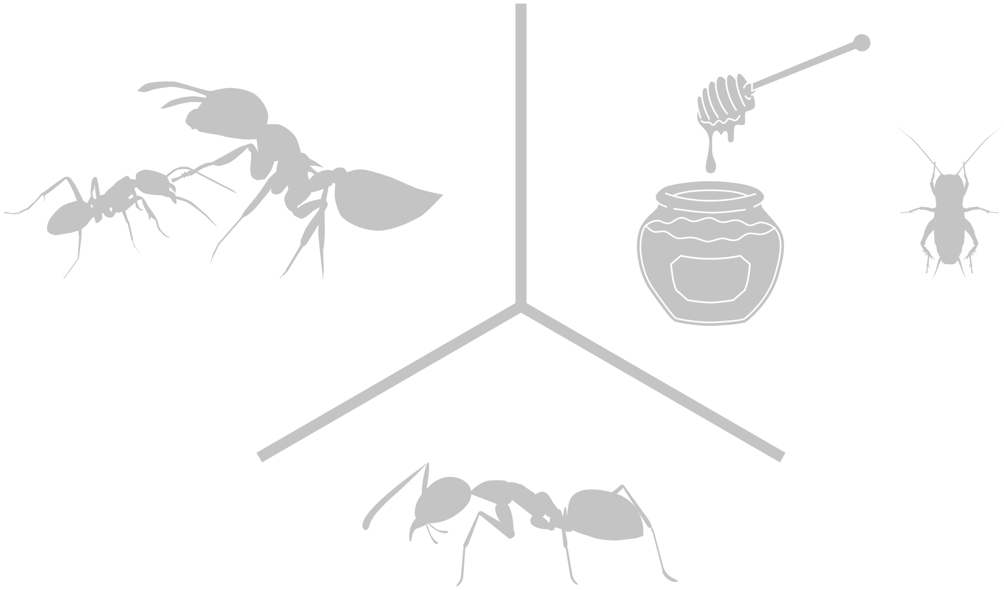


Figure S2. Relationships between the size of the cork oak tree with ant species richness (a) and ant abundance (b). In (a) trunk perimeter and ant richness correspond to the year 1992 when all these trees were uninvaded (but they have been distinguished by their invasion status in 2000). In (b) trunk perimeter and ant abundance correspond to the year 2016. Ant abundance was estimated as the number of ants per minute, counted in the thickest vertical ant trail of each tree. Data comes from invaded (red circles) and uninvaded (light blue diamonds) trees; in (b) data corresponds to the abundance of Argentine ants and native ants, respectively. Native ants measured were *Crematogaster scutellaris* and *Lasius grandis* (N = 16 and N=2 respectively). We show the statistics for the correlation tests performed, which were done with the *cor.test* function, in the 'stats' package of R (Core Team 2015).



CAN THE SUCCESS OF ARGENTINE ANT INVASION BE DETERMINED BY BIOTIC RESISTANCE AND PRESENCE OF EMPTY NICHES IN NATIVE COMMUNITIES?

Can the success of Argentine ant invasion be determined by biotic resistance and presence of empty niches in native communities?

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ABSTRACT

The Argentine ant is a highly invasive species spread over the globe, but in some locations it is unable to succeed completely in the invasion process, coexisting among native species without displacing them. In this study, we assess whether differences in the invasion success of Argentine ant across peninsular and insular Spain are related to differences in the invasibility (availability of empty niches) and/or the vulnerability (lack of biotic resistance) of native recipient ecosystems. We also tested whether variation in interspecific aggressiveness among Argentine ant colonies was correlated with invasion success, i.e. whether less aggressive colonies failed to displace the native ant community in unsuccessfully invaded areas. For this purpose, we compared 22 pairs of plots in locations hosting successful or unsuccessful Argentine ant invasions. At each location we sampled with pitfall traps ant communities from invaded and uninvaded (control) plots with a paired design, following a space-for-time substitution approach in which control plots were taken to represent native communities before the invasion. We did not find evidence of biotic resistance: native ant communities from control plots showed comparable richness, diversity, structure and proportion of dominant species was comparable within each pair of successfully and unsuccessfully invaded localities. We did not find evidence of the empty niche hypothesis either: removal rates of experimental baits (diluted honey and insect carcasses) of native ant communities from control plots was comparable within each pair of successfully and unsuccessfully invaded localities, indicating that the invasion was not facilitated by the existence of empty niches. However, we detected differences in the composition and structure of ant communities of successfully and unsuccessfully invaded locations: in the former, invaded plots showed larger decreases in species richness and diversity (as compared to control plots) than in the later. Finally, the aggressiveness of Argentine ants was larger at unsuccessfully invaded locations (as compared to successfully invaded ones), indicating that interspecific abilities were not causing invasion failure. This study contributes to the knowledge of what factors can be contributing to the success of Argentine ant invasion, showing the importance of comparisons between successfully and unsuccessfully invaded ant communities.

KEY WORDS

Linepithema humile, biotic resistance, aggressiveness, vacant niche, behavioral dominance, mass recruitment.

1. INTRODUCTION

The Argentine ant (*Linepithema humile*) is a global invader in ecosystems with Mediterranean-like climates (Suarez et al. 2001). Like in other invasive species, its success is based on certain life history traits. The small body size and high number of queens per nest facilitates transport by humans, increasing the likelihood of a fast geographic expansion (Bertelsmeier et al. 2017). The high number of workers per nest and generalist diet facilitates resource capture, increasing local dominance (Passera 1994; Walters & Mackay 2005). The lack of intraspecific aggression between colonies that are physically separated ('unicoloniality'; Silverman & Brightwell 2008) favors inter-colony cooperation and invasion spread (Vogel et al. 2010). Finally, the highly inter-specific aggressivity usually results in the complete displacement of native species from the recipient environments (Inoue & Goka 2009).

The Argentine ant is generally considered as a highly successful invader whose impacts are always severe. But a detailed examination of all its invasion areas show that they don't necessary abide by this description (e.g. Wetterer et al. 2006). In some areas, Argentine ant has been present for decades without displacing native species (Mothapo & Wosler 2014; Chapter I) or spreading to neighboring areas (Wetterer et al. 2006). This suggests that, despite its worldwide spread, there are factors that may limit its invasion success. Knowledge of these factors may be of key importance in preventing future invasions or managing ongoing ones.

For each given invasive species, a combination of suitable abiotic and biotic conditions is required to ensure ecosystem invasibility. For Argentine ant, extreme (high and low) temperatures and low moisture compromise its performance (Holway et al. 2002; Krushelnycky et al. 2005; Menke & Holway 2006; Menke et al. 2007), restricting its invasion potential to certain climatic regions (e.g. Roura-Pascual et al. 2011). Within such regions, small-scale variation in temperature, moisture, soil or microhabitat further determines its invasion success (Way et al. 1997; Thomas & Holway 2005; Menke et al. 2007). But, even under suitable abiotic conditions, biotic components could act by themselves, facilitating ('biotic facilitation') or hampering ('biotic resistance') the invasion process (e.g. Rowles & O'Dowd 2009; Blight et al. 2010). Understanding how native species contribute to invasion success or failure could greatly enhance the current predictions of the potential of invasion by the Argentine ant.

Biotic resistance against Argentine ant invasion may arise from interspecific competition, a process known to shape ant communities across the globe (Hölldobler & Wilson 1990), making native ant community composition a key determinant of its invasion success. Indeed, resistance against Argentine ant invasion has been reported to vary broadly among recipient ecosystems, from those showing no evidence of it (e.g. coastal scrubland in Doñana, south-west Spain, Castro-Cobo et al. 2019; riparian woodlands in California, USA, Holway 1998; coastal southeastern Australia, Rowles & O'Dowd 2007) to others where the dominant native species delayed its expansion (e.g. *Tapinoma nigerrimum* group in Corsica, Blight et al. 2010; *Iridomyrmex* genus in Australia, Thomas & Holway 2005, Walters & Mackay 2005, but see Rowles & O'Dowd 2007). In yet another cases, biotic resistance was provided by a previously established invader (e.g. *Pheidole megacephala* in South Africa, Mothapo & Wossler 2014). Ant species known to provide biotic resistance seems to be ecologically dominant (Chapter IV), with stronger competitive abilities such as mass recruitment (species that recruit large number of nestmates to exploit a resource, in comparison with group recruiters or solitary foragers; Lanan 2014) or behavioral dominance (species that monopolize resources, initiate attacks or trigger avoidance behavior in other species; Cerdá et al. 1997; Lach et al. 2009; Arnan et al. 2012). Identifying the species whose presence in native ant communities confers biotic resistance may therefore help explain the observed variation in Argentine ant invasion success.

Argentine ant, however, are not passive respondents to biotic resistance. Like many other invasive species, it can modify its competitive behavior in face of some species due to phenotypic plasticity. Argentine ants colonies have been reported to modify its behavior depending on relative competitor abundance, being more evasive when they are outnumbered (avoidance or escape strategy) and being more aggressive when they are more abundant (Carpintero & Reyes-López 2008; Sagata & Lester 2009). This behavioral plasticity in response to the number of competitors is also known as the 'bourgeois strategy' (Maynard Smith 1982) and allows the invasive species to deal with other numerically dominant species.

As described above, interspecific competition works through a combination of direct displacement (aggression, interference competition) and indirect competition (monopolization of trophic resources by natives or invaders, exploitation competition; Holldobler & Wilson 1990). An extreme case of the latter, which takes places in communities impoverished in species (e.g. islands, mountain tops) is the availability of empty niches: deserted whose resources are readily available for the (generalist) invasive species to exploit (Herbold & Moyle 1986; Mack et al. 2000; Ricciardi et al. 2013). Some of the most exploited food resources by the Argentine ant are carcasses of dead insects (Angulo et al. 2011) and honeydew produced by hemipterans (e.g. aphids, scale insects, mealybugs) (LeVan & Holway 2015). Incipient invasions of the Argentine ant need high protein levels to succeed in their invasion, while they tend to rely on a honeydew based diet once the colony is well established (Tillberg et al. 2007). While the presence of aphids is known to facilitate Argentine ant's invasion success (Grover et al. 2007), there is less information on whether competition by natives exploiting this resource modulates such effect. As for carcasses availability and removal, no study to date evaluates its impact on Argentine ant invasion success.

The aim of this study was to assess whether differences in the invasion success of the Argentine ant are caused by differences in the invasibility (availability of key resources, particularly in empty niches) and vulnerability (lack of biotic resistance) of native recipient ecosystems; or to a decreased aggression capacity of certain Argentine ant colonies due to behavioral differences among populations. For this purpose, we compared 22 pairs of plots in 10 locations hosting successful or unsuccessful Argentine ant invasions (where this species became abundant and displaced other ant species or remain less abundant and co-exist with other ants). At each location we sampled invaded and uninvaded (control) plots with a paired design, following a space-for-time substitution approach in which control plots were taken to represent native communities before the invasion.

First, we evaluated how the composition and the structure of the native ant community affected to the likelihood of invasion success (or failure), by comparing data from the control plots of successfully and unsuccessfully invaded locations. Following biotic resistance hypothesis, our hypothesis was that communities that in origin were richer, more diverse, or had higher proportion of dominant or mass-recruiter species, would have a higher chance of remaining unsuccessfully invaded. Second, we estimated the impact of invasion success on community structure, by comparing data from invaded plots of successfully and unsuccessfully invaded locations. We expected unsuccessfully invaded locations to maintain a community structure more similar to the original, native community. Third, we tested the empty niche hypothesis, by comparing the removal rate of insect carcasses and diluted honey in the control plots of successfully and unsuccessfully invaded locations. We expected a lower rate of bait removal by native species in control plots of successfully invaded locations. We also hypothesized that, in plots where there were more behaviorally dominant and mass recruiter native species feeding on these elements, Argentine ant invasion would be less successful. Fourth, we tested whether Argentine ants from unsuccessfully invaded plots, where they coexist with native species, were more or less aggressive than those from successfully invaded ones. Two alternative hypotheses could be possible: decreased aggressiveness in unsuccessfully plots could explain invasion failure, while increased aggressiveness in unsuccessfully plots could be a response to the constant competition with native ants.

2. MATERIAL AND METHODS

2.1. Study areas

We searched Argentine ants in different locations across peninsular and insular Spain where we were aware of Argentine ant invasion (Fig. 1a,b). We sampled locations where invasion was already reported, to prevent sampling sites with invasions in the first stages of the process (Table S1). We looked for sites with similar environmental characteristics, suitable for Argentine ant invasions: semi natural areas with dense pine forest but close to human settlements. Within each site, we selected localities with presence of Argentine ant and we made sure that

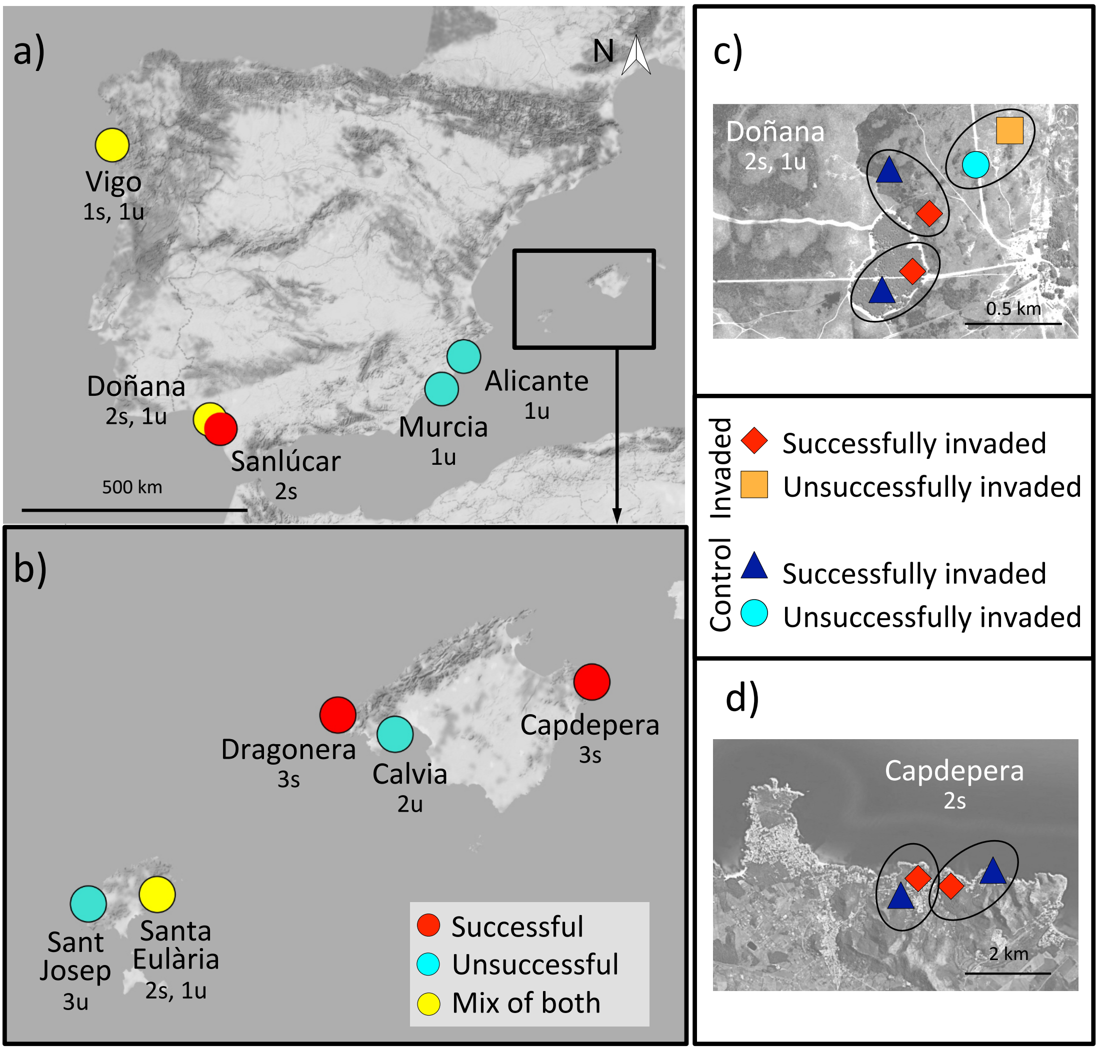


Figure 1. Map of the localities where the paired plots were situated in a) the Iberian Peninsula and b) the Balearic Islands. Two examples, one of each area, are showed in (c) where the paired design is showed by encircling paired invaded and uninvaded plots. In (a) and (b) areas with successfully invaded paired-plots are shown in red circles, unsuccessfully invaded appear in turquoise circles, and localities with a mix of successfully and unsuccessfully paired-plots are shown in yellow circles. Number of successful (s) and unsuccessful (u) paired-plots at each locality are marked.

the Argentine ant belonged to the Main supercolony through aggression test (for protocol, see Gomez & Espadaler 2005 and Chapter I). At each locality, we selected two sampling plots: one within an invaded area (invaded plot) and another in a nearby, ecologically similar, uninvaded area (control plot, Fig. 1c). Each pair of invaded-uninvaded plots (hereafter referred to as paired plots) followed a space-for-time substitution approach, whereby control (uninvaded) plots are intended to represent the original, native ant community of their paired invaded plot. Study areas were set in the following locations of the Spanish Peninsula (9 locations) and Balearic Islands (13 locations; number in parenthesis indicate the number of pairs of invaded-uninvaded plots set at each location; Fig. 1): Alicante (1), Calvia (2), Capdepera (2), Doñana (3), Dragonera (3), Murcia (1), Sanlúcar de Barrameda (2), Santa Eulària des Riu (3), Sant Josep de Sa Talaia (3) and Vigo (2). We aimed at using three locations (i.e., three pairs of plots) per sampling site, but owing to logistic and availability constrains, we only achieved this goal in 4 of the 10 sites surveyed.

2.2. Sampling native ant communities

To sample the ant community at each location, we set a series of 20 pitfall traps at each paired plot, in two transects of 100 m: one transect with 10 traps at the invaded plot and another at the uninvaded plot. Traps consisted in plastic recipients (7.5 cm diameter, 8 cm depth) filled with water and soap (to break surface tension). After 48 hours, trap contents were collected and preserved, separately for each trap, in 70% alcohol for their posterior identification in the laboratory. Individuals of each species were counted, to characterize the composition and structure of ant communities. Invaded plots where Argentine ants were observed abundantly in situ and had displaced most native species (<3 native species present) were categorized as 'successfully invaded plots'. Plots where Argentine ants was less numerous and co-existed with more native species were considered 'unsuccessfully invaded plots'. Successful and unsuccessful invasions were easy to detect when visiting locations, as signs were conspicuously visible, allowing us to obtain a similar sample size of both types of invasions. Nevertheless, we corroborated our classification a posteriori with data obtained in pitfall traps (Fig. S1).

2.3. Biotic facilitation and empty niche

To test the biotic facilitation hypothesis, we compared carcasses and honey bait removal by the Argentine ant and the native species. To estimate carcasses removal we set, at each paired plot, one transect of 10 points separated by 1 m intervals, where we placed (at 10 cm from each other) dead crickets of three different sizes: small ones (<5 mm), easily carried by a single ant; medium (11-15 mm), for ant species with group recruitment; and large (>21 mm), for ant species with mass recruitment. This represented a total of 30 crickets per plot and 60 crickets per paired plot. To estimate the removal rate of sugary liquid baits we set, at each paired plot, one transect of 10 points separated by 1 m intervals, where we placed one plastic spoon with diluted honey. Both types of baits (crickets carcasses and honey) were set in the morning (at approx. 8:30 am UTC +2, timeframe of high ant activity) and inspected four times: 30, 60, 120 and 180 minutes after setting the baits. At each observation, ant species identity, number of ant individuals per species and interference by other no-ant species were recorded. Samples of ant species were collected and kept in 70% alcohol for subsequent confirmation of the identification in the laboratory.

2.4. Ant aggressiveness

To characterize Argentine ant aggressiveness, we collected Argentine ant individuals from invaded plots and native species from uninvaded plots at all locations (i.e. for paired plots of both successfully and unsuccessfully invaded locations). For the tests, we selected 10 intact individuals (no visible physical damaged or odd behavior) of both the target native ant species and the Argentine ant. If the native species was polymorphic, we set 9 minor and 1 major workers. Individuals of both species were set at the same time in a vial tube (38 mm diameter x 65 mm long, with Fluon on the walls to avoid escaping) and they were observed continuously for 5 minutes, recording all aggressions from one species to the other. After five minutes, ants were let unobserved (still together at the same dish) for 55 minutes and then inspected to register the number of individuals dead, alive or injured (if one of the limbs was missed or the individual acted erratically) per species.

Table 1. Number of transects analyzed at each data analysis section.

Location	**Unsuccessful**		**Successful**	
Plot	**Uninvaded**	**Invaded**	**Uninvaded**	**Invaded**
Native ant community	10	10	12	12
Cricket baits	8	8	11	11
Honey baits	4	5	7	6
Aggression test	-	5	-	7

2.5. Data analysis

All data were analyzed with R (R Studio version 1.0.136, RStudio Team 2016). The number of paired plots used at each analysis varied according to the localities sampled for it (Table 1).

To characterize native ant community composition, we calculated species richness (S) and diversity values (Shannon index) for each transect, using the diversity function from vegan package (Oksanen et al. 2016). After that, we compared if invaded and uninvaded plots differed in richness, diversity or the proportion of behavioral dominant species or mass recruiters. For this purpose, we fitted two separate generalized linear mixed models (GLMMs) using the *glmmadmb* function (package glmmADMB, Fournier et al. 2012), type of plot (invaded vs. control) as a dependent, categorical variable, a binomial error distribution and a logit link function. In the first model, the independent variables were diversity and richness. In the second model the independent variables were the proportion of behaviorally dominant species and the proportion of mass recruiters. In both models, locality was included as a random factor to take into account the covariance of paired plots. The same procedure was followed to compare successfully vs. unsuccessfully invaded plots and separately, their control plots.

For the analysis of native ant community structure, we constructed a matrix of species abundance. We examined differences in ant community structure between: i) invaded vs. uninvaded plots, ii) invaded plots of successfully vs. unsuccessfully invaded localities, and iii) control plots of successfully vs. unsuccessfully invaded localities. We performed a permutational multivariate analysis of variance (PERMANOVA) using the *adonis* function (package Vegan, Oksanen et al. 2016). To observe the differences graphically, we performed a non-metric multidimensional scaling test (NMDS), using the *metaMSD* function (package vegan, Oksanen et al. 2016). We chose the appropriate number of dimensions by examining the stress value (correct below 0.15).

In order to test the empty niche hypothesis, we compared the removal rate of cricket carcasses between i) invaded vs. uninvaded plots, ii) invaded plots of successfully and unsuccessfully invaded localities, and iii) control plots of successfully vs. unsuccessfully invaded localities, by fitting three separated Cox regression model using the *coxme* function (coxme package, Therneau 2019) and we tested the significance of results with using the *Anova* function (car package, Fox & Weisberg 2011).

We also compared the discovery time of carcasses and the type of ants that discovered them by fitting three different models with structures as described above: invaded vs. uninvaded plots, invaded plots of successfully vs. unsuccessful invasions, and control plots of successful vs. unsuccessful invasions. The independent variables were discovery time and a variable that represented the degree of dominance of the ant species discovering the carcasses, with the following codes: 4 for behaviorally dominant ants that mass recruit, 3 for behaviorally dominant ants that do not mass recruit, 2 for behaviorally subordinate ants that are group recruiters, and 1 for behaviorally subordinate ant that are solitary foragers. We performed generalized mixed-effect models using *glmmadbm* function (package glmmADMB, Fournier et al. 2012) with a binomial error distribution and a logit link function. Transects were included as a random factor.

We also analyzed differences in the ants present in the carcasses between: i) plots invaded vs. uninvaded, ii) plots invaded successfully and unsuccessfully, and iii) their control plots (successfully and unsuccessfully). The independent variables were ant occurrence (number of carcasses occupied by ant per transect), the average number of ants at carcasses per transect and the proportion of species with behavioral dominance and with mass recruitment. We performed a generalized mixed-effect model using the *glmmadbm* function (package glmmADMB, Fournier et al. 2012) with a binomial link distribution and paired plots as random factors.

In the case of honey baits, we analyzed first whether the number of ants consuming the bait (accumulated through time) was affected by the type of plot (invaded vs. uninvaded) and the success of the invasion (successful or unsuccessful), including also time as a covariate (four times: 30 minutes, 1hour, 2 hours and 3 hours after the baits were set up). We included in the model all the interactions between the three independent variables and localities as a random factor. Because the dependent variable did not follow a normal distribution, we performed a generalized mixed-effect model using the *glmmadmb* function (package glmmADMB, Fournier et al. 2012). Second, we analyzed whether the maximum number of ants per bait present at each transect and the final number of ants present at each bait at the end of the survey were affected by the invasion status (invaded/uninvaded), the success of the invasion (successful/ unsuccessful) or their interaction. We modeled these variables as in the previous case, but in this case the random factor was the paired plot.

For the aggression tests, we analyzed three different dependent variables for both either native species or the Argentine ant (6 variables in total): the number of attacks to the opponent species during the first 5 minutes of the experiment, the number of dead individuals at the end of the trial, and the number of alive individuals at the end of the trial. As none of these variables followed a normal distribution, we performed generalized mixed-effect model using the *glmmadmb* function (package glmmADMB, Fournier et al. 2012) to analyze how the success of the invasion (successful/unsuccessful) of the area the Argentine ant came from, and the behavioral dominance of the native species facing it (dominant/ subordinate) affected the dependent variables tested. To consider covariance between species, they were included as a random factor in the models. We performed the same procedure but using only trials between the Argentine ant and the native *Crematogaster scutellaris*, a behaviorally dominant species (Arnán et al. 2014). In *C. scutellaris'* models, location was included as random factor (only 10 of the 12 localities were included, because this species was not present in 2 of the localities sampled for these trials: Sanlúcar de Barrameda and Vigo).

When the model residuals did not follow a gaussian distribution, as in the case of honey baits and aggression tests analyses, we chose the error distribution for count data that best fitted the observed residuals' distribution and optimized the model's fit – choosing among the Poisson, negative binomial and quasipoisson distributions. We fitted all the models with each one of the previous distributions and selected the one with best residuals' structure and the lowest AIC value, obtained with *AICtab* function (package bbmle, Bolker et al. 2017). In all cases, quasipoisson provided the best fit and ensured lack of residuals' overdispersion. Statistical differences for all generalized mixed-effect models described above were obtained with the function *Anova* (package car, Fox & Weisberg 2011).

3. RESULTS

Thirteen of the 22 localities sampled hosted invaded successful Argentine ant invasions, whereas in the remaining 10 the invasion was not successful: the Argentine ant coexisted with the native community. We found 44 native ant species in total in the pitfall traps (Table S2), with 1.62±0.40 (mean ± SE, hereafter) native species per plot for successfully invaded areas and 4.10±0.57 species for unsuccessfully invaded areas (Fig. S1). Abundance of the Argentine ant in the invaded plots was higher in successfully invaded localities, as compared to unsuccessfully invaded ones (173.39±98.12 vs. 12.33±3.63 individuals/plot,

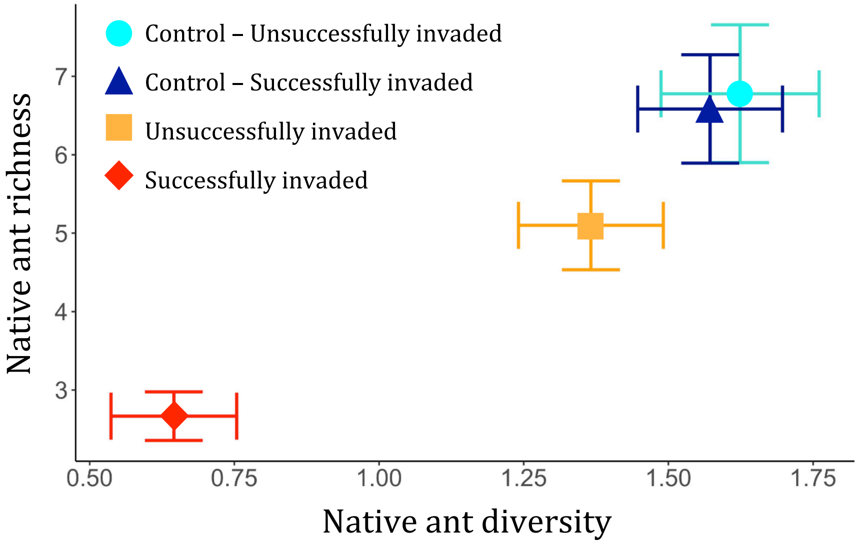


Figure 2. Diversity (X-axis) and species richness (Y-axis) of plots successfully invaded (red diamonds), their control plots (dark blue triangles), unsuccessfully invaded (orange squares) and their control plots (light blue circles)

Table 2. Effects of invasion status and success of the invasion according to (a) diversity and richness of the ant community; (b) Percentage of behaviorally dominant species and percentage of mass recruiters; and (c) ant community structure (Adonis). We present statistics (χ^2 values, p values and sample size, N) for the differences between invaded and uninvaded plots; in control plots between successfully invaded plots and unsuccessfully invaded plots, and in invaded plots between successfully invaded plots and unsuccessfully invaded plots.

	Invaded/Uninvaded			Control Successful/Unsuccessful			Invaded zones Successful/Unsuccessful		
(a)	χ^2	p	N	χ^2	p	N	χ^2	p	N
Richness	0.15	0.703	43	0.11	0.737	21	0.03	0.870	22
Diversity	**5.05**	**0.025**	**43**	0.17	0.677	21	2.26	0.133	22
(b)									
Beh. dominance	**3.90**	**0.048**	**43**	0.30	0.584	21	0.77	0.380	22
Mass recruitment	**4.65**	**0.031**	**43**	0.01	0.947	21	0.07	0.792	22
(c)									
Adonis	**8.76**	**0.001**	**44**	0.77	0.726	22	**3.76**	**0.008**	**22**

respectively, Fig. S1). The abundance of native ants in the uninvaded, control plots was, in contrast, comparable between unsuccessfully and successfully invaded localities (7.09±0.90 vs. 4.83±5.94 individuals/plot).

3.1 Native ant community composition and structure

Native species richness did not differ significantly between invaded and uninvaded plots, but diversity (as measured by the Shannon index) was significantly lower at invaded plots (Table 2a; Fig. 2). Species richness and diversity did not differ significantly between successfully or unsuccessfully invaded localities, neither for invaded nor for uninvaded plots (Table 2a; Fig. 2). Invaded plots had significantly less proportion of behaviorally dominant species and mass recruiters than uninvaded plots (Table 2b), but these variables did not differ significantly between successfully and unsuccessfully invaded localities (neither for invaded nor for uninvaded plots; Table 2b).

Community structure differed significantly between invaded and uninvaded plots, as well as between the invaded plots of successfully and unsuccessfully invaded locations, but not between the control plots of both location types (PERMANOVA, Table 2c). The NMDS test with three dimensions resulted in fair stress values (of 0.09, 0.15 and 0.08 respectively) in all three cases. In the NMDS plots, ant community structures were clearly differentiated between invaded and uninvaded plots (Fig. 3a) and overlapped between successfully vs. unsuccessfully invaded locations (Fig. 3b-c) – although, for the invaded plots, it showed a reduced spread for successfully invaded locations, as compared to unsuccessfully invaded ones (Fig. 3c).

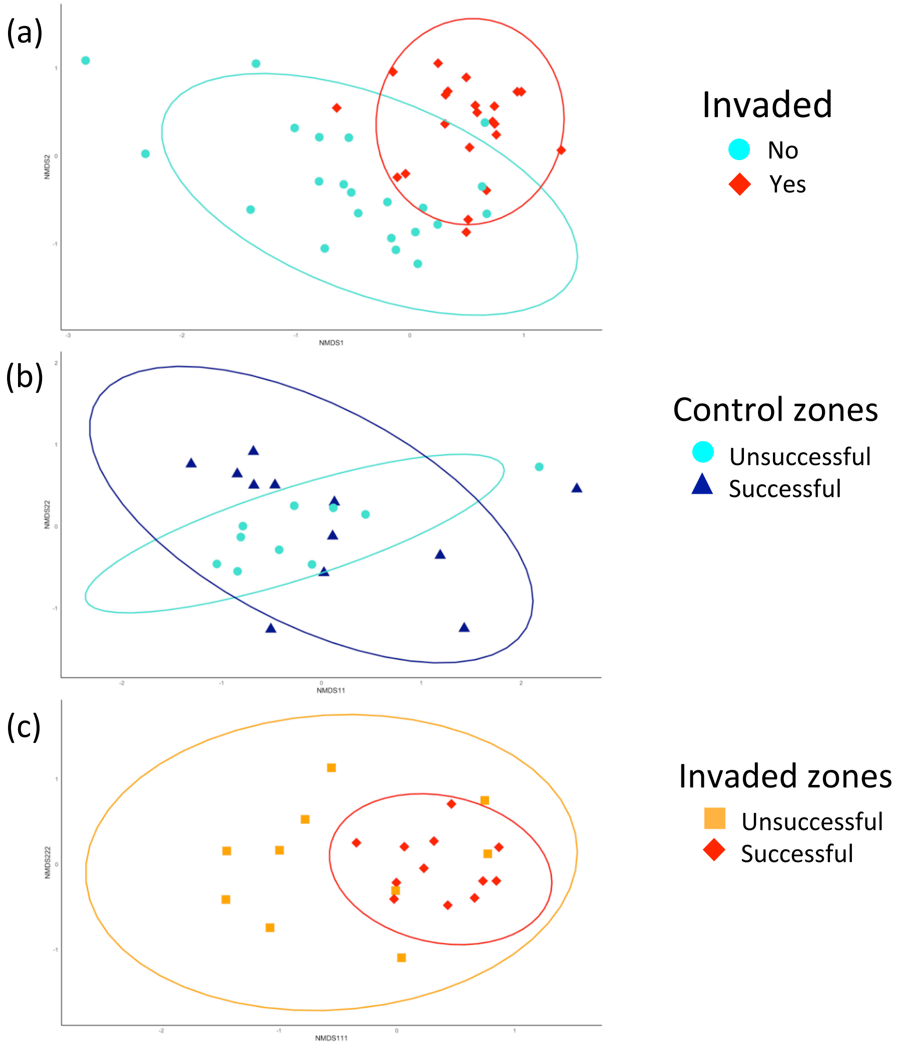


Figure 3. Ordination of ant community similarity (NMDS) for (a) invaded (red diamonds) and uninvaded, control (turquoise circles) plots, placed using a paired design within localities hosting Argentine ant invasions; (b) control plots within either successfully invaded (dark blue triangles) or unsuccessfully invaded (turquoise circles) locations; and (c) invaded plots within either successfully invaded (red diamonds) or unsuccessfully invaded (orange squares) locations. Ellipses represent 95% confidence intervals for each group.

Table 3. Effects of invasion status and success of the invasion on foraging activity of ant communities at experimental death crickets. (a) Removal rate of crickets along time; (b) Discovery time of the crickets by ants, and dominance type of the ant that discovered the cricket (combining the behaviorally dominance and the ability to mass recruit); (c) Number of crickets occupied by ants per transect (occurrence), proportion of behaviorally dominant ant species, proportion of mass recruiters and average number of ants in the crickets per transect (model of areas invaded successfully vs. unsuccessfully did not converge). We present statistics (χ^2 values, p values and sample size, N) for the differences between invaded and uninvaded plots; in control plots between successfully invaded plots and unsuccessfully invaded plots, and in invaded plots between successfully invaded plots and unsuccessfully invaded plots.

	Invaded Uninvaded			Control successful Control unsuccessful			Invaded successful Invaded unsuccessful		
(a)	χ^2	p	N	χ^2	p	N	χ^2	p	N
Removal rate	0.00	0.952	1118	0.02	0.880	559	1.34	0.247	559
(b)									
Discovery time	0.01	0.946	279	0.01	0.942	130	0.01	0.918	149
Dominance type	0.14	0.709	279	0.01	0.907	130	0.00	0.977	149
(c)									
Occurrence	**3.77**	**<0.001**	31	0.04	0.843	15	-	-	-
Prop. beh. dom.	**1.03**	**<0.001**	31	0.00	0.967	15	-	-	-
Prop. mass recruit.	**2.94**	**<0.001**	31	0.00	0.967	15	-	-	-
Number of ants	6.51	0.799	31	0.01	0.916	15	-	-	-

3.2 Empty niches: carcasses and honey consumption

Neither carcasses removal rate nor discovery time nor dominance type (of the ant species that discovered the bait) differed significantly between invaded and uninvaded plots, or between successfully and unsuccessfully invaded localities (for both invaded and control plots; Table 3a, Fig. 4).

Invaded plots had significantly more carcasses occupied by ants, mostly Argentine ants. In those carcasses with native species, they included more mass recruiters but less behaviorally dominant species (Table 3c). All other comparisons were not significant or could not be tested because models failed to converge.

None of the three variables measuring ant foraging at sugary baits was significantly affected by plot type (invaded vs. control), locality type (successfully vs. unsuccessfully invaded) or their interaction (Table 4, Fig 5).

Table 4. Effects of invasion status and success of the invasion on foraging activity of ant communities at experimental honey baits through time. Foraging activity is measured using: (a) the accumulated number of ants at each honey bait along time, (b) the final number of ants at honey baits per transect, and (c) the maximum number of ants at honey bait per transect. We present statistics (χ^2 values, p values and sample size, N).

		(a) Accumulated number of ants		(b) Final number of ants		(c) Maximum number of ants	
	N	χ^2	p	χ^2	p	χ^2	p
Time	88	**9.72**	**0.002**	-	-	-	-
Invasion	88	0.22	0.636	0.17	0.685	0.16	0.686
Success	88	0.20	0.654	0.39	0.532	2.64	0.104
Time*invasion	88	0.12	0.733	-	-	-	-
Time*success	88	0.01	0.923	-	-	-	-
Invasion*success	88	0.63	0.429	0.18	0.668	0.04	0.844
Time*invasion*success	88	1.06	0.304	-	-	-	-

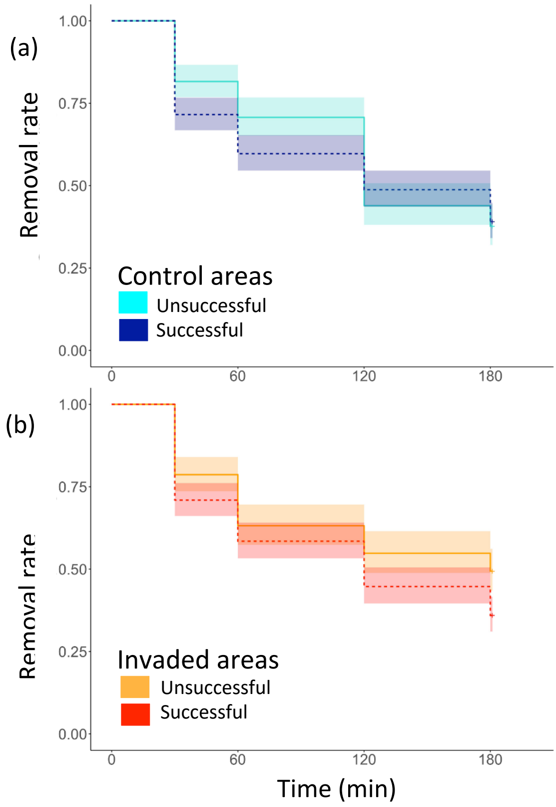


Figure 4. Removal rates of cricket carcasses during 180-minutes trials at: (a) uninvaded, control plots situated within either successfully invaded locations (dark blue) or unsuccessfully invaded locations (light blue); and (b) invaded plots situated within either successfully invaded locations (red) or unsuccessfully invaded locations (orange).

3.3 Aggression tests

Invasion success (at the locality of origin of the Argentine ant used in each aggression test) had a significant effect on all but one of the variables measuring the outcomes of the aggression tests (Table 5), while the behavioral dominance of the native species used in the test had a significant effect on none of them (Table 5). Argentine ants from successfully invaded performed significantly more.

Aggressions than those from unsuccessfully invaded plots and, probably as a consequence, had less individuals alive at the end of the trials (Fig. 6c,d).

Similarly, in the aggression tests using *C. scutellaris*, those facing Argentine ants from unsuccessfully invaded plots performed significantly more aggressions but showed a lower survival at the end of the trial (Fig. 6e, f; Table 5).

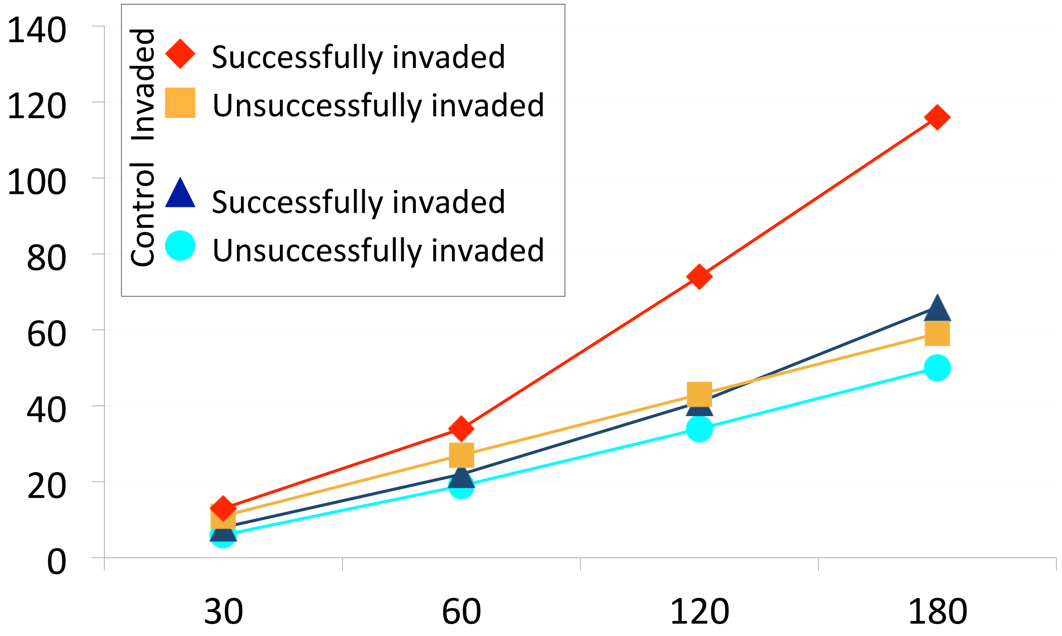
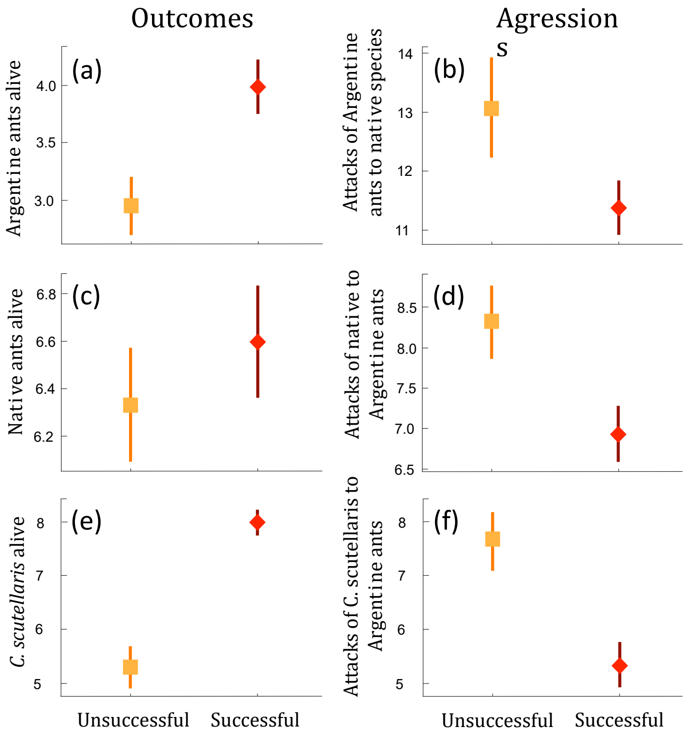


Figure 5. Accumulated number of ants foraging at honey baits during 180-minutes trials, in invaded and uninvaded control plots situated within either successfully invaded or unsuccessfully invaded locations.

Table 5. Differences in the outcomes and aggressions in confrontations between the Argentine ants versus all native species, or the Argentine ant versus *Crematogaster scutellaris* specifically, a behavioral dominant species very common in our study areas. Outcomes are represented by the number of dead and alive workers of Argentine ant or native species (or *C. scutellaris*) at the end of the trial (1h); aggressions are represented by the number of attacks realized by each of the species during the firsts 5 minutes of the trial (Arg -> Nat, attacks performed by the Argentine ant; Nat -> Arg, attacks performed by native ants). S indicates the sign of the relationship; we modeled the probability of success.

	Behavioral dominance				Success				Success (*C. scutellaris*)			
	F	p	N	S	F	p	N	S	F	p	N	S
N arg alive 1 h	0.40	0.527	279	-	**4.64**	**0.031**	**279**	**+**	1.46	0.226	95	+
N arg dead 1 h	0.67	0.414	279	+	**8.06**	**0.005**	**279**	**-**	0.55	0.461	95	-
N nat alive 1 h	3.15	0.076	279	-	**12.74**	**<0.001**	**279**	**+**	**12.56**	**<0.001**	**95**	**+**
N nat dead 1 h	3.52	0.061	279	+	0.18	0.670	279	+	1.91	0.167	95	-
Arg -> Nat (5 min)	0.17	0.683	279	+	**12.60**	**<0.001**	**279**	**-**	2.80	0.094	95	-
Nat -> Arg (5 min)	0.42	0.520	279	+	**7.98**	**0.004**	**279**	**-**	**5.20**	**0.023**	**95**	**-**

Figure 6. Outcomes and aggressions when confronting 10 Argentine ants and 10 individuals of a native species from either successfully invaded (red diamond) or unsuccessfully invaded (orange square) locations: (a) number of Argentine ants alive; (b) number of attacks of Argentine ants to native ants; (c) number of native ants alive; (d) number of attacks of native ants to Argentine ants; (e) number of *Crematogaster scutellaris* ants alive, and (f) number of attacks of *C. scutellaris* ants to Argentine ants. Symbols represent mean values, bars standard errors. Number of ants was counted at the end of the 1h-trial, while number of attacks was counted during the first 5 minutes of the trial. Data of (a) to (d) come from separate trials with 12 different native species pooled).



4. DISCUSSION

We studied invasibility and vulnerability of native recipient ecosystems to the invasion of the Argentine ant through different approaches, using a space-for-time substitution approach at 22 different localities of Spain (Peninsula and Balearic Islands), with the main aim of finding differences between successful invasions as opposed to unsuccessful invasions where the invader failed to become dominant. We did not found evidence in support of the hypothesis of biotic resistance – i.e., neither the species richness, diversity and structure of the native ant community in uninvaded plots (assumed to represent the ant community before the invasion), nor the proportion of behaviorally dominant and mass recruiting species in such communities, were significantly correlated with the probability of a successful invasion by Argentine ant. We did not find either evidence in support of the empty niche hypothesis – i.e., in the absence of Argentine ants (control, uninvaded plots assumed to represent the pre-invasion stage), native species from successfully and unsuccessfully invaded locations were equally good at exploiting insect carcasses and diluted honey. In contrast, we found a significant correlation between invasion success and Argentine aggressiveness, which was higher in unsuccessfully invaded locations than in successfully invaded ones. Such higher aggressiveness was associated with a higher mortality during the aggression trials, in both the native and the invasive species. Further work is required to explore the causality behind this relationship, which may either reflect a higher likelihood of success when the initial invaders were more aggressive and thus increasing their likelihood of success or a posterior strategy to cope with constant competition with native species in unsuccessfully invaded areas.

Argentine ant invasion had a severe impact on native ant communities, which (as expected) was stronger in successful invasions – with unsuccessfully invaded locations showing intermediate values of diversity and community composition. This gradient in community changes suggests that, in unsuccessfully invaded localities, the Argentine ant could be integrated as part of the ant community (Chapter I, Chapter IV). Functional parameters of the community, such as carcasses and bait removal, were seemingly unaffected. Several studies have described how the Argentine ant displaced native species at invaded areas, reducing ant richness drastically (Ward 1987; Cammell et al. 1996; Suarez et al. 1998; Sanders et al. 2003; Touyama et al. 2003). But, as we show here, this is not always the case: the Argentine ant was not able to invade successfully at almost half of the sampled localities, even after decades of reported presence through the coast of the Iberian Peninsula (Table S1). Moreover, we decided to sample these 22 original locations where the Argentine ant was reported without knowing a priori if there were successful or unsuccessful invasions, resulting in almost half of the locations with unsuccessful invasions.

In previous literature, situations in which the Argentine ant coexisted with native species were generally influenced by abiotic factors (e. g. Holway et al. 2002; Carpintero et al. 2003). Temperature has been seen to affect coexistence of native species with the Argentine ant in two different ways. Low temperatures allow species with higher cold tolerance, such as *Prenolepis imparis* or *Pachycondyla chinensis*, to segregate their seasonal and/or daily activity peaks, facilitating co-existence with the Argentine ant (Fitzgerald & Gordon 2012; Spicer Rice & Silverman 2013). Conversely, high temperatures (especially if combined with low moisture) can favor thermophilic native species, allowing them to outcompete Argentine ants (Menke et al. 2007; Angulo et al. 2011; Thomas & Holway 2005) or to coexist through the use of non-overlapping activity patterns (Angulo et al. 2007). In addition, certain soil types, like sandy and clay loam, can prevent spread of the Argentine ant in agricultural systems, allowing other dominant species to co-occur with it (Way et al. 1997). Some of these factors may have played a role in the (lack of) success at the invaded locations sampled.

The Argentine ant shows high aggressiveness against native species (Ward 1987; Holway 1999; Human & Gordon 1999). Specifically, it is very aggressive towards species that are ecologically similar to it, i.e. mass recruiters, behaviorally dominants and generalists (Carpintero et al. 2007; Abril & Gomez 2009). Interestingly, these ecologically-similar species are often those excluding the Argentine ant spread at some regions, like *P. pallidula, C. scutellaris* and *T. nigerrimum* in Portugal (Way et al. 1997), *Lasius grandis* in Madeira (Wetterer et al. 2006), and *T. nigerrimum* in Corsica (Blight et al. 2010). In our aggression tests, we did not find evidence that the Argentine ant was more aggressive to dominant native species than to subordinate ones, but interestingly we found differences in aggressions according to the success of the invasion. These results contrast with those reported by Abril and Gomez (2009), who did not find differences in terms of aggressiveness of Argentine ants confronting the behaviorally subordinate native *Plagiolepis pygmaea* from areas where species were in contact and not, but are in agreement with Sagata and Lester (2009), who suggested that behavioral plasticity could have a role in Argentine ant success.

The availability of food resources rich in carbohydrates, like honeydew, has been shown to promote both the aggressiveness of invasive ants (such as Argentine ant; Grover et al. 2007) and the likeliness of a successful invasion (Helms & Vinson 2002; Abbot & Green 2007; Brightwell & Silverman 2011). Specifically, the availability of these food resources helps Argentine ants to displace native species (Rowles & Silverman 2009; Kay et al. 2010) and to survive out of its suitable niche (Brightwell & Silverman 2011). At pine forests, like our study areas, aphids constitute an important food resource for ant species that attend them for their honeydew, or predate on them or on their predators (Mooney & Tillberg 2005). Contrary to our expectation, native species in control areas of unsuccessfully and successfully invaded plots proved to be as good as the Argentine ant at removing these resources. Therefore, our results suggested that the invasion success was not related to the empty niche hypothesis, at least for the food resources we examined here.

Using a broad survey of 22 pairs of invaded-uninvaded plots across insular and peninsular Spain this is the first study that compares successful and unsuccessful invaded ant communities by the Argentine ant. We show that neither biotic resistance nor empty trophic niches were likely to mediate invasion success of Argentine ants in the western Mediterranean. Behavioral variation, on the other hand, may have mediated such success, by adjusting aggression levels to the presence of native competitors. Long-term monitoring of invaded ecosystems is essential to discern the combination of factors that allow recipient communities to prevent or favor the spread of invasive species.

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SUPPLEMENTARY MATERIAL

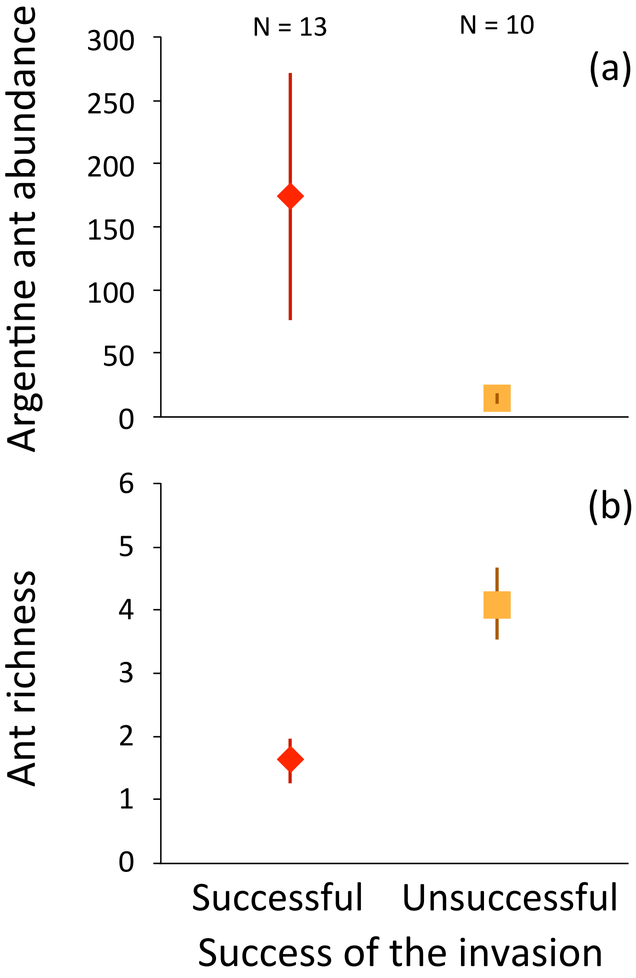

Figure S1. Mean (SE) of Argentine ant abundance (a) and number of native species (b) in plots successfully and unsuccessfully invaded. Differences in abundance and in the presence of native ant corroborate visual classification of successfully and unsuccessfully invaded areas.

Table S1. List of locations and plots, success of the invasion, sampling date and date from which Argentine ant invasion is known for each location (First det.) and references. Some references correspond to members of the Iberian Association of Myrmecology (AIM). Coordinates of each plot (Coord) and the success of the Argentine ant invasion (Success) are marked.

Location	**Coord (lat, long)**	**Success**	**# Paired plots**	**Date**		**References***
				Sampled	First det.	
1. Vigo	42.150716° -8.838701°	U, S	2	07/2017	2003	Gerardo Fernández, AIM
2. Doñana	36.990456° -6.4509	U, S	3	07/2015	1980's	Carpintero et al. 2003
3. Sanlúcar de Barrameda	36.847345° -6.316010°	S	2	09/2017		
3. Murcia	37.600639° -1.035931°	U	1	07/2016	2010	Chema Catarineu, AIM
4. Alicante	38.206721° -0.510340°	U	1	07/2016	2011	Javier Arcos, AIM
5. Capdepera	39.703700° 3.456098°	S	2	07/2015	1988	Espadaler & Gómez 2003
6. Calvia	39.533268° 2.585938°	U	2	06/2015	1988	Espadaler & Gómez 2003
7. Dragonera	39.588911° 2.329054°	S	3	07/2015	2003	Espadaler & Gómez 2003
8. Santa Eulària	38.977155° 1.530700°	U, S	3	07/2015	2004	Espadaler & Gómez 2003
9. Sant Joseph	38.950188° 1.228687°	U	3	07/2015	2004	Gomez & Espadaler 2005

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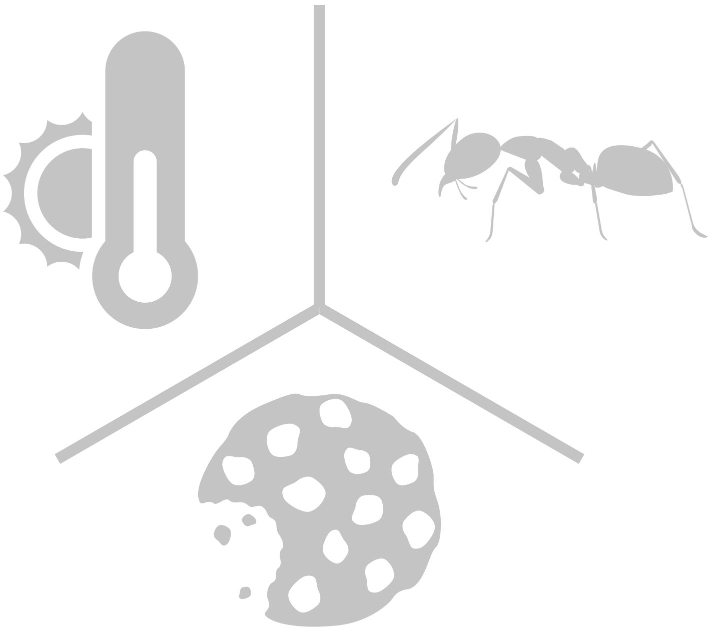
Table S2. List of the species found in the pitfall traps. The range of distribution is categorized in Iberian (I) or Andalousian (A) endemisms, or invasive species (V). Abundance corresponds to the number of plots with presence of each species from a total of 22 paired plots (44 plots in total), and in brackets is the total number of ants. Location numbers correspond to locations in Fig. 1.

Species	Range	Abundance	Locations
Aphaenogaster gibbosa		2 (11)	1
Aphaenogaster senilis		7 (152)	3
Aphaenogaster iberica	I	2 (16)	1
Camponotus lateralis		6 (24)	4
Camponotus ruber		5 (49)	4
Cardiocondyla mauritanica	V	1 (1)	1
Cataglyphis floricola	A	2 (50)	1
Cataglyphis iberica	I	1 (1)	1
Cataglyphis tartessica	A	1 (313)	1
Colobopsis truncatus		1 (3)	1
Crematogaster auberti		3 (50)	3
Crematogaster laestrygon		3 (403)	2
Crematogaster scutellaris		18 (287)	8
Crematogaster sordidula		2 (54)	1
Formica fusca		2 (57)	1
Hypoponera eduardi		2 (2)	1
Iberoformica subrufa	I	1 (201)	1
Lasius grandis		2 (66)	1
Lasius niger		2 (3)	1
Linepithema humile	V	32 (2685)	10
Messor bouvieri		6 (80)	3
Messor lusitanicus		1 (76)	1
Monomorium subopacum		1 (4)	1
Myrmica aloba		2 (65)	2
Myrmica ruginodis		2 (92)	1
Myrmica scabrinodis		2 (98)	1
Pheidole pallidula		12 (147)	7
Plagiolepis pygmaea		18 (92)	8

Plagiolepis schmitzii		10 (36)	5
Ponera sp		1 (1)	1
Solenopsis spp		7 (22)	4
Tapinoma erraticum		1 (1)	1
Tapinoma madeirense		4 (20)	3
Tapinoma nigerrimun cfr		3 (12)	2
Temnothorax angustulus		1 (3)	1
Temnothorax lichtensteini		1 (1)	1
Temnothorax nylanderi		1 (1)	1
Temnothorax pardoi		3 (20)	2
Temnothorax racovitzai		12 (138)	4
Temnothorax recedens		19 (291)	6
Temnothorax specularis		4 (8)	3
Temnothorax tyndalei		3 (5)	2
Tetramorium caespitum cfr		3 (151)	3
Tetramorium forte		1 (5)	1
Tetramorium semilaeve		12 (320)	5



COMPETITIVE INTERACTIONS BETWEEN NATIVE SPECIES AND THE INVASIVE ARGENTINE ANT ACCORDING TO BEHAVIORAL DOMINANCE AND FORAGING STRATEGY

Competitive interactions between native species and the invasive Argentine ant according to behavioral dominance and foraging strategy

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ABSTRACT

Invasion success depends on biotic and abiotic characteristics of the recipient ecosystem. The Argentine ant, *Linepithema humile*, is very harmful invader, but its success can be compromised when competing against native ant communities in stressful abiotic environments, and traits of native species related to competitive performance could help to determine the outcome of these interactions. We analyzed the competitive abilities of the Argentine ant and four native species that locally co-occur in a relatively arid environment for the invader. Native species differed in their combinations of two traits: behavioral dominance and ability to recruit. We assessed competitive performance using bait monopolization observations and short-term removal experiments. We also surveyed literature to determine how other species that co-occur with the Argentine ant in other locations expressed these traits. Foraging performance of all our studied native species was reduced when competing with the Argentine ant, being the most affected the behaviorally dominant ones. Argentine ant foraging activity was compromised the most by species that mass recruited, although competition with behaviorally dominant species was also important. Although our findings were based in a small number of species, the literature review supported them. 56% of ant species reported to co-occur with the Argentine ant were mass recruiters and behaviorally dominant. Our results showed that in interspecific competition, the Argentine ant affected and was affected by species holding specific life history traits, and the degree of the effect could depend on these traits. These results deepen our understanding of the mechanisms involved in competitive interactions between native and invasive ants.

KEY WORDS

Interspecific competition, foraging activity, *Linepithema humile,* behavioral dominance, mass recruitment, *Solenopsis xyloni*.

1. INTRODUCTION

The Argentine ant (*Linepithema humile* (Mayr 1868)) is an invasive species that has successfully invaded areas with Mediterranean-like climate around the globe (Suarez et al. 2001). By displacing native ant species, it disrupts native communities (Holway et al. 2002a; Sanders et al. 2003), with cascading effects in the ecosystem such as seed dispersal lost (Rowles & O'Dowd 2009); plant growth and pollination reduction by tending honeydew-producing hemipterans more effectively than native ants (LeVan & Holway 2005; Nygard et al. 2008); decrease in diversity and abundance of other invertebrates (Krushelnycky et al. 2008); or declines and spatial shifts of small vertebrates including amphibians (Alvarez-Blanco et al. 2017), reptiles (Suarez & Case 2002) and birds (Boieiro et al. 2018).

Traits that contribute to the Argentine ant to invade new environments include the ability to form supercolonies, aggressive behavior, polygyny, large colony size, and being able to establish successfully with relatively small propagules (Passera 1994; Hee et al. 2000). Moreover, the physical environment limits where the Argentine ant can invade (Holway et al. 2002b; Menke & Holway 2006; Menke et al. 2007). However, less is known about the biotic factors that mediate invasion of novel ecosystems (Zenni & Nuñez 2013), limiting our ability to prevent future invasions or identify riskier ecosystems. Although native ant richness does not repel the invasion (Holway 1998, Sanders et al. 2003), abiotic and biotic factors may interact enhancing the ability of native ants to interfere with the invasion (Holway et al. 2002; Thomas & Holway 2005; Wetterer et al. 2006; Menke et al 2007). In particular, our understanding of whether or not native ant species with different traits and competitive abilities can curb Argentine ant invasion is limited. Currently, predictions of the potential for Argentine ant invasion are primarily based on the effects of the abiotic environment alone (Holway et al. 2002b; Roura-Pascual et al. 2010), thus ignoring the potentially important role of interactions with native species (but see Menke et al. 2007).

Traits involved in competition among ants would be likely some of the most related in the co-occurrence of native species with the Argentine ant. Behavioral dominance often manifests itself in differences in food collection strategies and aggressiveness (Cerdá et al. 2013). Behaviorally dominant ant species tend to aggressively defend food resources, whereas behaviorally subordinate species avoid confrontation (Arnan et al. 2012; Cerdá et al. 1997; Lach et al. 2009). Competitive ability also depends on ant foraging strategies, with species varying from solitary foragers to those that exhibit group recruitment (e.g., up to some dozens of workers) or mass recruitment (e.g., large numbers of workers at baits) (Cerdá et al. 2013; Arnan 2014). Behavioral dominance, together with mass recruitment, allows ant species to forage more efficiently at resources (Cerdá et al. 1997, 1998; Arnan et al. 2012). This could be of great importance at locations where abiotic conditions are not completely suitable for the Argentine ant. In these conditions, native species could provide higher competition to the invader and thus make more difficult its spread (Holway et al. 2002b). However, it is unknown how precisely these traits could play a role in the invasion of the Argentine ant.

In this study, we compared the foraging ability and competitive interactions between the Argentine ant and four native ant species selected to vary in behavioral dominance and mass recruiting behavior. First, we characterized bait monopolization of Argentine ant and the native ant species where they interact across a moisture gradient with the dry end of this gradient serving as a refuge for native ants (Holway 2005; Menke & Holway 2006). We then conducted reciprocal, short-term removal experiments to test how foraging activity of the Argentine ant is compromised in the presence of native competitors and how competition with the Argentine ant reduces the foraging activity of those species. We hypothesized that, under these environmental conditions, native species opposing more resistance to the Argentine ant would be the ones that mass recruit and are behaviorally dominant. For that, we used four native ant species that differed in these traits. We interpret our results with respect to the native ant species' behavioral dominance and capacity to recruit. Finally, we performed a literature review aimed at quantifying the frequency at which behavioral dominance and mass recruiting were associated with native and invasive Argentine ant co-occurrences.

2. MATERIALS AND METHODS

2.1. Study area

This study was conducted in Dawson Los Monos Canyon Reserve in San Diego County (33° 9' 3.39" N, 117° 14' 54.94" W; California, USA). Fieldwork was performed from July to September 2017 in the northern part of the reserve (Fig. 1). This study site encompassed an expanse of shrub habitat adjacent to a riparian woodland (primarily supporting *Quercus agrifolia* and *Platanus racemosa*). The Argentine ant occurs abundantly in the riparian area ("invaded area" in Fig. 1) but decreases in abundance away from it ("uninvaded area" in Fig 1). Native ants overlap with the Argentine ant in the scrub zone ("area of overlap" in Fig. 1), and this area of contact between native ants and the Argentine ant has been present

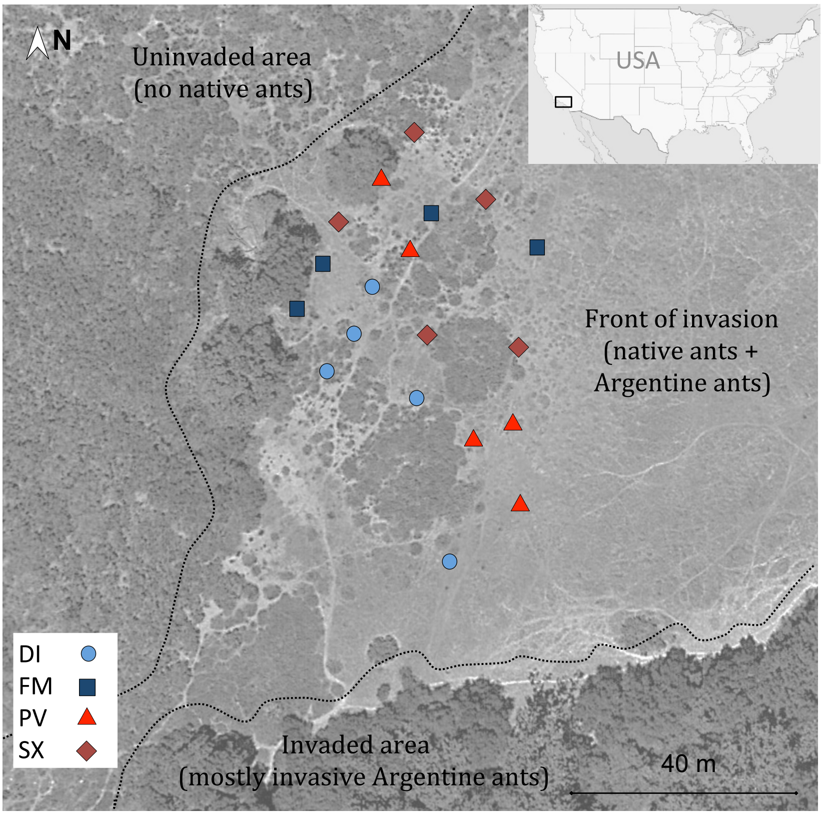


Figure 1. Map of the study area, showing the front of invasion where the sampling points for each native species were located, as well as the invaded and the uninvaded areas. DI: *Dorymyrmex insanus,* FM: *Formica moki,* PV: *Pheidole vistana*, SX: *Solenopsis xyloni.*

for over a decade (Menke & Holway 2006). All the experiments of this study were performed in the area of overlap.

2.2. Species selection

We assessed the competitive abilities of four native ant species: *Dorymyrmex insanus* Buckley, 1866; *Formica moki* Wheeler, 1906*; Pheidole vistana* Forel, 1914; and *Solenopsis xyloni* McCook, 1879*.* These species occur commonly in chaparral and coastal sage scrub ecosystems in San Diego Co., CA. We categorized the behavioral dominance and recruitment strategy of each species based on personal observations and reports in the literature (Table 1): *Solenopsis xyloni* is behaviorally dominant (Menke et al. 2010) and mass recruits (*personal*

Table 1. Life history traits of ant species considered in this study. Information was derived from literature, the AntProfiler database ([1]) (http://www.antprofiler.org), and personal observations ([2]). LH: *Linepithema humile*, SX: *Solenopsis xyloni, PV: Pheidole vistana, FM: Formica moki, DI: Dorymyrmex insanus.* Literature cited correspond to: [3]Arnan et al. 2012, [4]Holway et al. 2002b, [5]Lanan 2014, [6]Menke et al. 2010, [7]Schilman et al 2007, and [8]Wittman et al. 2010. Question marks represent traits for which we have doubts.

A	DomB	Recruit	WS	P	CS	Nnest	NQ	Diet	Def	Attack	Unic
LH	D[2,3]	1[2,3]	2.86[2]	1[2,3]	3[3]	P[3]	P[3]	H/I[3]	2[3]	PC[3]	1[3]
SX	D[2,6]	1[2]	3.05/3.41/5.40[2]	3[2,7]	3	F[3]	P[1]	H/I[6]	2[3]	PC[2]	0[2]
PV	S[2]	1[2]	3.05/5.14[2]	3[2]	2[1]	P[2]	P[1]	I[2]	1[2]	P[2]	0[2]
FM	D[2,8]	0[2]	-	2[2]	2?[2]	P[2]	P?[2]	H/I[2]	2[2]	PC[2]	0[2]
DI	S[2]	0[2,4,5]	3.32[2]	1[2]	1[1]	F[2]	P[1]	I[3]	1[2]	P[2]	0[2]

B

DomB	Behavioral dominance: D: dominant, S: subordinate
Recruit	Type of foraging strategy: 1: mass recruiter, 0: other kind or foraging
WS	Mean worker length (mm)
P	1: monomorphism, 2: low polymorphism, 3: high polymorphism
CS	Colony size: 1: hundreds, 2: thousands, 3: tens of thousands.
Nnest	Number of nests per colony: P: polydomy, M: monodomy, F: facultative
NQ	Queens per colony: P, polygyny, M: monogyny
Diet	Main food resource: H: Honey, I: Insects, S: Seeds
Def	Defense of resources: 1: none, 2: food and/or territory.
Attack	P: physical attack, C: chemical attack, PC: both
Unic	Unicoloniality: 0: no, 1: yes

observations). *Pheidole vistana* is behaviorally subordinate and mass recruits (*personal observations*). *Formica moki* is behaviorally dominant (Wittman et al. 2010) and does not mass recruit; although it can recruit nestmates to food resources (Holway 1999), in our experiments it behaved as solitary forager. *Dorymyrmex insanus* is behaviorally subordinate and can recruit in high numbers (Holway 1999) or not (Lanan 2014), as is the case at our study area (*personal observations*).

2.3. Bait monopolization

We used baits to observe patterns of bait monopolization and foraging behavior of each of the five ant species included in this study. The use of baits in the field can provide insight into intraspecific and interspecific competitive interactions among ants (Cerdá et al. 1997; Cerdá et al. 2013).

On two occasions during the first two weeks of July, we set out 35 baits (cookies containing carbohydrates, fats and proteins that are attractive to all species in this study as we observed; Pecan Sandies, Kellog Inc, Battle Creek MI). From 7 am to 8 pm, baits were placed every 5-10 m along four parallel transects separated by 10 m, resulting in a 60 x 80 m grid. We recorded the number and identity of ants foraging at each bait four times per day: twice in the morning and twice in the evening. The morning and the evening were considered separate events of bait occupation, as baits had to be recolonized in the evening due to the high temperatures in the middle of the day, when no ants of any species were observed foraging. All the species included in the study were actively foraging during these periods of time the baits were set. Soil temperatures surrounding each bait were recorded during every observation. We considered a species to have monopolized a bait when it was the only species present during the final observation (of the morning or evening). We calculated bait monopolization for each species as the number of baits the species successfully monopolized, using all the observations.

2.4. Short-term removal experiment

Short-term removal experiments at baits provide a means to study interspecific competitive interactions (Holway 1999; Thomas & Holway 2005; LeBrun et al. 2007). Here we use this approach to compare the ability of two species to capture food in each other’s presence (control; neither species removed) with their ability to recruit to food when one of the competitors was excluded (removal treatment) (LeBrun et al. 2007). Foraging performance was estimated using three different parameters: time to discover the bait, time to recruit a group of workers, and maximum number of ants recruited in the bait. Discovery time and recruitment time are parameters related to exploitative ability (e.g., the ability of species to find and use resources before others (Cerdá et al. 2013). The recruitment response (e.g., the maximum number of ants at a bait) can be associated with the ability of a species to defend that bait from other species. We explored how the presence of the Argentine ant affected the foraging performance of each native species and *vice versa*.

Based on the patterns of bait monopolization, we selected areas where foraging activity of Argentine ants and the native species overlapped. These areas (hereafter referred to as plots, Fig. 1) were selected for each of the four native species in our study as follow: (i) when we found that the Argentine ant was foraging within 0.5 m of the native species, and (ii) the plot was at least 1 m away from a nest entrance of any species. We selected five plots for each native species, with the exception of *F. moki* for which there were four due to the difficulty in locating this species' nests.

Based on temperature and activity patterns that we recorded during the bait monopolization observations, we performed the short-term removal experiments in late afternoon, because all focal species were typically more active during this time. In each plot we placed a cookie bait (same type as in the bait monopolization observations). Individual cookies were only used for one day and then discarded. The short-term removal experiment consisted of three treatments that were performed for each pair of species over three consecutive days: treatment one, where all individuals of a particular native species were removed (through aspiration) before they could enter the plot, allowing only Argentine ant workers to forage on the bait; treatment two, where all individuals of the Argentine ant were removed, allowing only the native species that we were studying at that plot to forage on the bait; and treatment three as a control, with no removal, so individuals of both species were allowed to compete for the bait. The order of species, plots, and treatments and control was randomly selected.

The experiment ran for three hours starting with the moment that the bait was set. During the experiment, we registered the discovery time, the recruitment time (time to recruit 10 workers), and the number of ants at the bait every 15 minutes. Soil temperature was taken at the beginning of the experiment and also every 15 minutes until the end of the experiment, when the bait was first discovered, and when the focal species recruited 10 workers. Ants were also collected from baits to confirm their identity in the laboratory.

2.5. Data Analysis

All analyses were conducted in R (R Studio version 1.0.136, RStudio Team 2016). In bait monopolization analyses, we first ran a Chi square test with the *chisq.test* function (package stats, R Core Team 2017) to test whether the five ant species in our study monopolized different numbers of baits. Second, to test if species differed with respect to the number of baits they monopolized, we tested the null hypothesis that all species were the same (i.e., each monopolize 20% of the baits). Given that we expected to reject this null hypothesis, we used an exact test of goodness-of-fit per species with the *binom.test* function to assess whether not each species was above or below the null expectation (package stats, R Core Team 2017). Finally, we performed pair-wise post-hoc comparisons between species with the *pairwiseNominalIndependence* function and using Bonferroni corrections (package rcompanion, Mangiafico 2019).

To assess what parameters affected the ability of each species to monopolize baits, we ran separate generalized linear models with binomial distributions (0 = did not monopolize a bait, 1 = monopolized a bait) for each species using the *glm* function (stats package, R Core Team 2017). *Formica moki* was the only species we did not analyze because it only monopolized one bait. Some factors like temperature at which the bait was monopolized, time of day that the sampling was performed (morning or evening), or what day it was carried out (the 1st day of the trial or the 2nd one) could also affect monopolization. We thus included these factors in the models as independent variables to test how they influence monopolization. We used Chi square statistics to test for the significance of each factor with the *Anova* function (car package, Fox & Weisberg 2011).

In the analysis of the short-term removal experiment data we considered four different dependent variables: discovery time (the time required for a species to locate a bait), recruitment time (the time required for 10 workers to be recruited to a bait), the maximum number of workers present at the bait during the trial, and the difference over time in the accumulated number of workers (as described below). We analyzed this experiment following two approaches in order to examine separately the effects of competition (removal) on native ants and the Argentine ant: (i) the variation in the Argentine ant's foraging performance in response to the removal of native species, and (2) the variation in native species' foraging performance in response to the removal of the Argentine ant. To test these two approaches, we analyzed the dataset of the four native species together and, separately, the dataset of the Argentine ant.

Thus, to analyze what factors affected the dependent variables, discovery and recruitment time and maximum number of ants in the bait, we used Generalized Linear Mixed-Effects Models with the *glmmadmb* function (package glmmADMB, Fournier et al. 2012). We included in the analysis two main independent variables and the interaction between them: removal of the rival species (two categories, distinguishing whether the species was foraging alone as a consequence of the removal or whether competition occurred) and the native species for which the plot was selected (four categories: one for each native species; see Fig. 1). We added plot identity as a random factor to take into account covariance of measures of the same plot. For each dependent variable, we included a soil temperature covariate as follows: temperature at which bait discovery occurred (for discovery time models), when the first recruitment occurred (for recruitment time models) and mean temperature at which the maximum number of workers occurred (for the maximum number of workers models). We ran three models (one model per dependent variable) for the Argentine ant and three models for the native species together, totaling six models. Statistical differences for each model were obtained with the function *Anova* (package car, Fox & Weisberg 2011). Because none of the dependent variables followed a normal distribution, we constructed multiple possible Generalized Linear Mixed-Effects Models based on different distributions (Poisson, negative binomial or quasipoisson) for each dependent variable and selected best-fitting models by comparing AIC values using the *AICtab* function (package bbmle, Bolker et al. 2017). In all cases, the best-fitting models were those with the negative binomial distribution, except for the difference in the accumulated number of workers for which a quasipoisson distribution was used. None of the models with the best-fitting distribution were overdispersed, so those were the distributions used (Table S1).

Additionally, we examined how bait occupancy changed over 180 min in the presence of the competitor. We first calculated the number of accumulated workers at the bait across the 15 min observation intervals, by summing the number of workers recorded during the entire 180 min. We then calculated the difference between the accumulated numbers of workers at the bait every 15 min when the species was competing minus the accumulated number of workers when the species was alone. This difference reflects how the presence of a heterospecific affects recruitment by a focal species. Negative values indicate that the species performed worse in the presence of a competitor than it did by itself, and vice versa for positive values. To test this effect statistically, we performed two Generalized Linear Mixed-Effects Models with the *glmmadmb* function (package glmmADMB, Fournier et al. 2012). Similar to the previous analyses, we analyzed the data of the four native species together and, separately, the data of Argentine ant. The dependent variable for these two models was the difference in the accumulated number of workers, described above. The main independent variable was the native species for which the plot was selected (four categories: one for each native species). We added the plot identity as a random factor. Time was included as a covariate, and we were interested in the 'time*species' interaction, which revealed if species differed with respect to how recruitment responses changed over time. Statistical differences for each model were obtained with the function *Anova* (package car, Fox & Weisberg 2011).

For those dependent variables that differed across species or species interaction with removal (for discovery and recruitment time or maximum number of ants) or time (for the difference in the accumulated number of ants through time), we performed planned post hoc comparisons. Two planned comparisons were performed in order to test whether differences among species were associated with species that differed in their behavioral dominance or in their ability to recruit. First, grouping species by behavioral dominance (the subordinates *D. insanus* and *P. vistana* vs. the dominants *F. moki* and *S. xyloni*). Second, grouping species by recruitment type (the mass recruiters *P. vistana* and *S. xyloni* vs. the ones that did not mass recruit *D. insanus* and *F. moki*). We used *lsmeans* function when the independent significant variable was the species (lsmeans package, Lenth 2016); and *Anova* function when the independent significant variable was the interaction of species with removal or species with time, using a factor grouping species by behavioral dominance, and a factor grouping species by recruitment type (package car, Fox & Weisberg 2011).

2.6. Literature review

We performed a literature review to determine the frequency that behavioral dominance and recruitment types were associated with ant species co-occurring with the Argentine ant. We identified ant species that differed in their susceptibility to be displaced by the Argentine ant, and had available information of the targeted traits.

We used three search strategies. First, we performed a bibliographic search at the FORMIS database (http://www.ars.usda.gov/saa/cmave/ifahi/formis), using the words "*Linepithema humile*" and "biotic resistance" as key terms. Second, we used the Global Ant Database (globalants.org, Gibb et al. 2017) to search ant species that co-occurred in the same ant community with the Argentine ant. Third, additional relevant materials were found as cited by the analyzed documents. To ensure this search was not biased by the lack of species not co-occurring with the Argentine ant, we searched in the associated paper, or in others from the same authors, specific species lost due to the invasion.

To get the life history traits for the focal species we used the database ANT PROFILER (Fournier et al. 2019), searched information in the literature, and required information from experts (provided them with the definition of our traits as stated in the introduction section).

3. RESULTS

3.1 Bait monopolization

From a sample size of 148 baits (32 + 42 baits, twice a day), 21 were not recruited to by any species and 10 were occupied by more than one species, resulting in a total of 117 baits that were monopolized by one of the five species. Presence of the species at baits was registered as follows: *D. insanus* was present in 66 baits, *F. moki* in 5, *P. vistana* in 57, *S. xyloni* in 70, and the Argentine ant in 90.

Solenopsis xyloni monopolized the greatest number of baits (40 baits), followed by the Argentine ant (39 baits), *P. vistana* (26 baits), *D. insanus* (11 baits), and *F. moki* (1 bait), respectively. Thus, the Argentine ant and *S. xyloni*, the two ant species that were behaviorally dominant and mass recruiters, significantly monopolized higher numbers of baits (Proportion of success = 0.333, $p < 0.001$ and Proportion of success = 0.342, $p < 0.001$ respectively), while *D. insanus* and *F. moki*, had significantly lower activity in monopolizing baits, and both lack mass recruitment, (Proportion of success = 0.094, $p = 0.003$ and Proportion of success = 0.009, $p < 0.001$ respectively). *Pheidole vistana*, which is behaviorally subordinate but also mass recruits, was the only species that colonized the percentage of baits that was not different from the null expectation (Proportion of

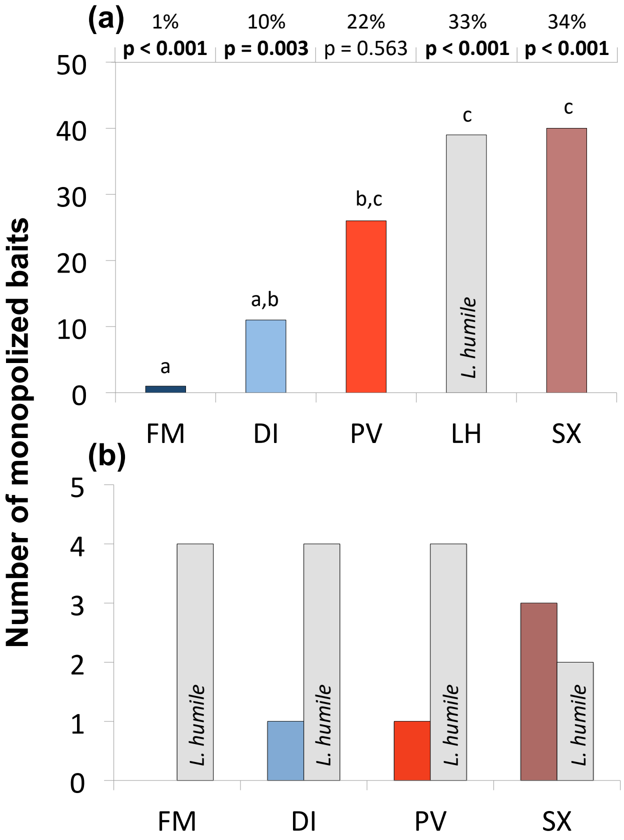


Figure 2. Number of baits monopolized by each species, (a) during the bait monopolization observations, and (b) in the short-term removal experiment. In (a) bars with different letters are significantly different (Table S2); the percentage of monopolized baits and the p-value from the exact test of goodness-of-fit is showed above. Total number of baits was 117 in (a) and 5 replicas in (b) except for *Formica moki* that was 4. DI: *Dorymyrmex insanus,* FM: *Formica moki,* PV: *Pheidole vistana*, SX: *Solenopsis xyloni,* LH: *Linepithema humile.*

success = 0.222, p = 0.563). The number of baits monopolized significantly differed across ant species ($\chi^2 = 63.10$, $p < 0.001$, N = 5; Fig 2a). Pair-wise comparison indicated that differences in monopolization among the Argentine ant, S*. xyloni* and *P. vistana*, were not significant ($p > 0.05$ for all the cases, Table S2), all mass recruiters. A similar result occurred for *D. insanus* and *F. moki*, both no mass recruiters (p = 0.076). Differences between species with different recruitment strategies were palliated by *P. vistana*, which at the same time was not significantly different in its level of bait monopolization from *D. insanus*; these two species were behavioral subordinates but differ in their recruitment type (p = 0.121, Fig 2a, Table S2).

Temperature at bait, day, and time of day (morning or evening) had different effects on bait monopolization of each species (Table S3). Temperature effect on *L. humile* and *D. insanus* was in opposite directions: monopolization was higher for *D. insanus* when temperature increased ($\chi^2 = 10.00$, p = 0.002, N = 117), while monopolization was higher for *L. humile* when temperature decreased ($\chi^2 = 13.45$, $p < 0.001$, N = 117). Time of the day was significant for *P. vistana* ($\chi^2 = 7.96$, p = 0.005, N = 117), with bait monopolization being lower in the morning than in the afternoon. The day that the sampling was done significantly affected the number of baits monopolized by *L. humile* ($\chi^2 = 20.36$, $p < 0.001$, N = 117), *P. vistana* ($\chi^2 = 5.62$, p = 0.018, N = 117) and *S. xyloni* ($\chi^2 = 30.21$, $p < 0.001$, N = 117). The Argentine ant monopolized more baits on the first day, and *P. vistana* and *S. xyloni* monopolized more baits on the second day.

3.2 Short-term removal experiment

We paired the four native species with the Argentine ant and observed the pairs either interacting at baits or each species foraging alone (when removing one of the opponents). When native species interacted with the Argentine ant, *L. humile* occupied the bait more successfully at the end of the 180 min trial, defeating the native species it was competing with (Fig. 2b). The only exception was *S. xyloni*, which was able to control the bait against the invader in three out of five trials (Fig. 2b). Throughout the baiting we obtained data on foraging activity (discovery and recruitment time, maximum number of workers at bait and accumulated number of workers at bait) for the five species and the three treatments (Figs. 3 and 4).

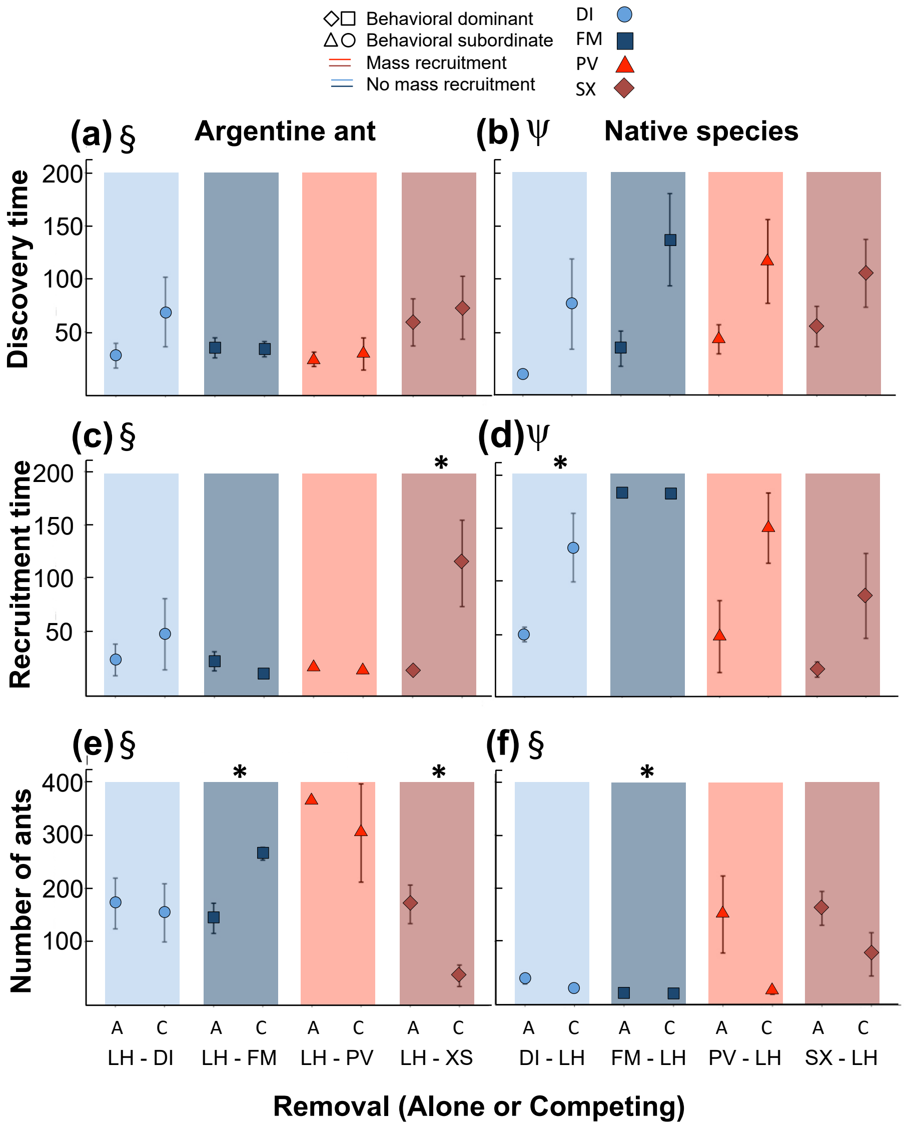


Figure 3. Foraging activity of the Argentine ant (left 4 columns) and native species (right 4 columns). Colored bars indicate the native species involved in the short-term removal experiments. Symbols inside the bars show the mean ± SE for the Argentine ant (left columns) or for each native species (right columns) when the species is foraging alone versus when they are competing (A and C, respectively, along the X-axis). Foraging activity at bait is represented with the discovery time (min), the recruitment time (min), the maximum number of ants in the bait and the number of accumulated ant at the bait through time. In the recruitment time only those trials with more than 10 workers are included. Variables with a significant interaction between the ant species and the presence of a competitor are marked with the symbol '§', while variables with a significant effect of the presence of a competitor are marked with 'Ψ'. Significant differences for species when competing vs. foraging alone are marked with an asterisk (see also Table S4). DI: *Dorymyrmex insanus,* FM: *Formica moki,* PV: *Pheidole vistana*, SX: *Solenopsis xyloni,* LH: *Linepithema humile.*

Discovery time

Discovery time by the Argentine ant was significantly affected by the interaction between the ant species and the removal (Table 2A), indicating that the effects of native ants differed across species. Mean discovery time increased more when the Argentine ant was in competition with *D. insanus* and *S. xyloni* (41 min and 14 min of increase, respectively) than when in competition with *F. moki* and *P. vistana* (1 min and 4 min of increase, respectively; Fig. 3a). Planned comparisons among species grouped by their level of behavioral dominance or recruitment type did not show significant differences ($p = 0.878$; $p > 0.726$ respectively). Increasing temperature significantly decreased discovery time of the Argentine ant (Table 2A).

In the case of native ants, discovery time significantly increased in the presence of the Argentine ant; but there were no differences among species or in the interaction between species and removal (Table 2A, Fig. 3a). Discovery time by native ant species also decreased with temperature (Table 2A). The species that, in the absence of the Argentine ant, had a shorter discovery time was *D. insanus*; the Argentine ant had intermediate values, and the species that took more time to discover the bait when foraging alone was *S. xyloni* (Table 3, Fig. 3a,b).

Recruitment time

Recruitment time by the Argentine ant was significantly affected by the presence of other species and the interaction with the removal (Table 2B). When we performed post hoc planned comparisons to assess whether or not these differences were associated with the traits of the native ants, we found no significant differences when grouping species by their behavioral dominance ($p = 0.126$), and a marginal effect when grouping species by mass recruitment type ($p = 0.078$). This finding may have been influenced by *S. xyloni*, which most increased the Argentine ant's recruitment time (from 14.20 ± 7.70 min to 114.20 ± 90.26) (Fig. 3c, Table S4).

In the case of native species, recruitment time did not significantly differ among species (Table 2B). However, heterospecific removal significantly decreased recruitment time of all native species (Fig. 3d). Recruitment time also increased significantly with decreasing temperature (Table 2B). The species that, in the absence of the Argentine ant, had a shorter recruitment time was *S. xyloni* (Table 3, Fig. 3d).

Maximum number of ants in the bait

The maximum number of Argentine ants at the bait was significantly affected by both the presence of the heterospecific and its interaction with removal (Table 2C). Mean maximum number of ants decreased when the Argentine ant interacted

Table 2: Effects of native species on foraging parameters of the Argentine ant and native species: (a) discovery time, (b) recruitment time and (c) the maximum number of ants in the bait and (d) number of accumulated ants through time. Interactions of species with removal treatment (for (a), (b) and (c)) and time (for (d)) are shown.

	Argentine		**Natives**	
	χ^2	p	χ^2	p
A. Discovery time				
1. Species	2.15	0.542	0.46	0.927
2. Removal	3.76	0.053	**4.56**	**0.033**
3. Temperature	**35.06**	**< 0.001**	**59.86**	**< 0.001**
4. Removal*Species	**10.57**	**0.014**	1.55	0.671
B. Recruitment time				
1. Species	**3.30**	**< 0.001**	2.85	0.416
2. Removal	2.63	0.105	**5.34**	**0.021**
3. Temperature	3.30	0.069	**4.70**	**0.030**
4. Removal*Species	**11.26**	**0.010**	3.77	0.288
C. Number of ants				
1. Species	**14.16**	**0.003**	**10.29**	**0.016**
2. Removal	1.47	0.226	1.89	0.169
3. Temperature	**21.46**	**< 0.001**	0.02	0.886
4. Removal*Species	**9.08**	**0.028**	2.82	0.420
D. Accumulated ants				
1. Time	1.72	0.190	**16.33**	**< 0.001**
2. Species	2.11	0.549	0.55	0.908
3. Time*Species	**29.51**	**< 0.001**	**19.22**	**< 0.001**

Table 3. Main characteristics of the ant species when foraging at baits alone, without a competitor. Nw/bait: mean maximum number of workers per bait registered. Discovery/recruitment times: mean discovery/recruitment times (minutes) for each species ± SE. Foraging behavior relates to the one observed in our experiments: mass recruitment, group recruitment or solitary foraging.

	Nw/bait	Discovery time	Foraging behavior	Recruitment time
LH	216	36.52 ± 31.88	Mass recruitment	19.16 ± 18.17
SX	162	54.60 ± 42.54	Mass recruitment	15.00 ± 15.95
PV	150	42.60 ± 30.65	Mass recruitment	45.80 ± 75.16
FM	1	33.75 ± 33.18	Solitary foraging	180*
DI	29	9.20 ± 6.80	Group recruitment	47.80 ± 15.24

* *Formica moki* did not recruit, so the time assigned is the total duration of the experiment, 180 min.

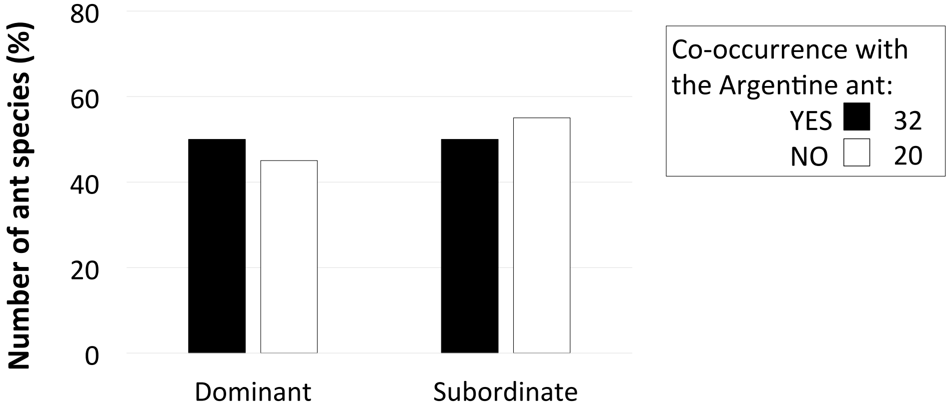
with *P. vistana* (from 364 to 304 ants) and *S. xyloni* (from 170 to 36), while it increased in the presence of *F. moki* (from 144 to 265) and remained relatively stable in the presence of *D. insanus* (from 172 to 154; Fig. 3e). Moreover, the increase in the presence of *F. moki* and the decrease in the presence of *S. xyloni* were both significant (Fig. 3e, Table S4). However, planned post hoc comparisons among groups of species, grouping them by behavioral dominance or recruitment type, did not show significant differences ($p = 0.979$ and $p = 0.278$, respectively). Increasing temperature significantly depressed the maximum number of ants in the bait.

For the focal native species, the maximum number of workers present at baits differed across species (Table 2C). Higher numbers of workers were observed for mass recruiting species than for species that did not recruit in large numbers (Fig. 3f). In fact, planned post hoc comparisons revealed a significant effect when grouping species by their recruitment time ($p = 0.008$), but no effect when grouping them by their behavioral dominance ($p = 0.994$). Although the maximum number of ants at baits was reduced in the presence of the Argentine ant (23% less for *S. xyloni*, 50% for *D. insanus*, and 90% for *P. vistana;* Fig. 3f), the effects of removal or its interaction with species were not significant (Table 2C).

Accumulated number of ants through time

As seen in Fig. 4, both the Argentine ant and the native species recruited fewer

(a) Behavioral dominance



(b) Mass recruitment

(c) Mass recruitment & behavioral dominance

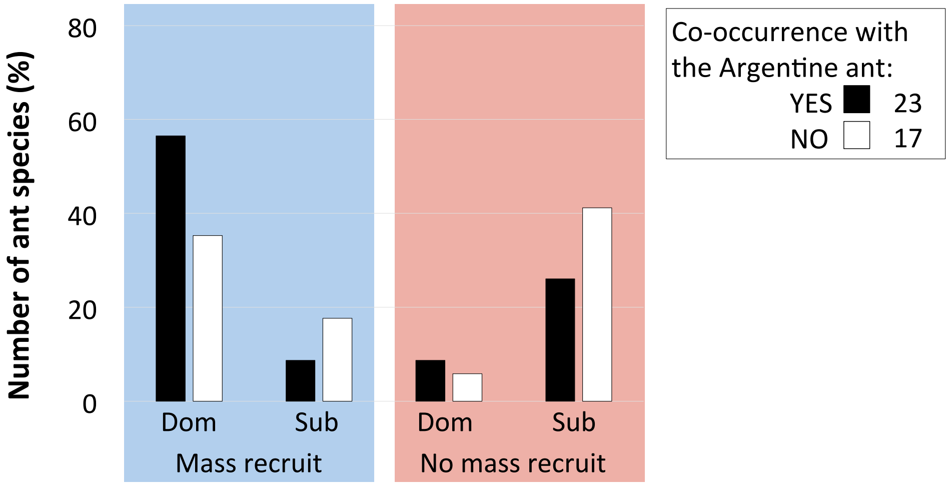


Figure 4. Difference (mean ± SE) in the number of ants in the bait due to competition (*competing – alone* treatments). In (a) differences are shown for the Argentine ant with each native species. In (b) differences are shown for each native species' number of workers.

workers to baits in the presence of a heterospecific than alone, and this effect was stronger for native species than for the Argentine ant (Fig. 4a *vs.* Fig. 4b). The numbers of Argentine ant workers at baits over time differed with respect to the native species in question (Table 2D). Planned post hoc comparisons showed that mass recruiting species depressed Argentine ant recruitment over time ($p < 0.001$); no significant effect was found when native species were grouped by their behavioral dominance ($p = 0.096$). The presence of the Argentine ant also depressed native ant recruitment over time with native ant species varying in the extent to which recruitment was depressed (Table 2D). In contrast to the pattern for the Argentine ant, planned post hoc comparisons were significant when grouping species by their behavioral dominance ($p < 0.001$), but not when grouping species by their mass recruitment type ($p = 0.181$).

3.3 Literature review

We found information for a total of 55 species, 35 that co-occurred with the Argentine ant, and 20 that were susceptible to displacement by it. For most ant species, we obtained information about the two traits in our study, behavioral dominance and recruitment type (Table S5). We calculated the percentage of species that co-occurred with the Argentine ant or were sensitive to be displaced by it, and we related this information with the two life history traits (Fig 5).

Behavioral dominant species tended to co-occur in slightly higher proportion to the Argentine ant than subordinate species, while behaviorally subordinate species tended slightly more to be displaced (Fig. 5a). Mass recruiters usually co-occurred with the Argentine ant in higher proportion than ants with other recruitment strategies (63% vs. 50%, Fig. 5b). Species lacking mass recruitment tended to be displaced (50% vs. 35%, Fig. 5b).

Finally, when we combined ants with both traits, we saw that the highest percentage of ants co-occurring with the Argentine ant were mass recruiters with behavioral dominance (56% of species); while behaviorally subordinate ants that did not recruit had relatively high values of displacement by the Argentine ant (41%; Fig 5c).

4. DISCUSSION

We assessed the role of different native species in modifying the foraging ability of the Argentine ant when interacting at baits. In an area where native ants co-occur with the Argentine ant, we showed that the foraging performance of the Argentine ant was affected in different ways when interacting with these species. *S. xyloni*, species that mass recruited and was behaviorally dominant, decreased the number of Argentine ant workers at baits to a greater degree than when the invader interacted with the other species. Meanwhile, the presence of the Argentine ant negatively affected foraging activity of native species, although the activity of *P. vistana*, species that mass recruited and was behaviorally subordinate, decreased the most. Finally, the literature review showed how a high proportion of the species reported to co-occur with the Argentine ant were behaviorally dominant that mass recruited.

The outcomes of interspecific competition between invaders or with native species are influenced by life history traits of the species they are competing with (Hogg & Danee 2011; Perdereau et al. 2011; Gidoin et al. 2015). For example, in California vineyards different hunting modes and lower body size of native spiders resulted in lower competition of the native community against the introduced *Cheiracanthum mildei* Koch, 1839 (Hogg & Danee 2011). Competition for resources among wasps in *Megastigmus* genus can result in co-occurrence due to divergences in some life history traits (Gidoin et al. 2015). We discuss below how differences in competition-related traits in native ant assemblages could influence the outcome of competitive interactions of native species against the Argentine ant.

Mass recruitment and behavioral dominance have been cited as important traits that allow invasive ants to outcompete native species (Abbott et al. 2007; Rowles & O'Dowd 2007; Menke et al. 2018). But these same mechanisms could allow native species to defend their nests or food resources against the invasive ants, preventing the spread of the invasion. For example, *S. xyloni* in our study is a mass recruiter and behaviorally dominant species. Although it is widely outcompeted when abiotic conditions are more suitable for the Argentine ant, it also can occupy arid environments that are unsuitable for this invader (Menke et al. 2007). When *S. xyloni* competed against the Argentine ant in our experiments, the Argentine ant significantly lost foraging activity while *S. xyloni* was less affected. Our literature review showed how in other regions, ecologically similar species such as *Iridomyrmex rufoniger* (Lowne, 1865) in Australia, also competes effectively against the Argentine ant (Walters & Mackay 2005) while in Corsica *Tapinoma nigerrimum* (Nylander, 1856), which possess these two traits, seems to be limiting Argentine ant's expansion (Blight et al. 2010). However, we found also that *P. megacephala* (Fabricius, 1793) in Africa is sensitive to displacement by the Argentine ant (Mothapo & Wossler 2014), while in California another behaviorally dominant mass recruiter native species, the winter ant *Prenolepis imparis* (Say, 1836), using a powerful chemical defense is able to fight back effectively confrontations against the Argentine ant (Sorrels et al. 2011). But in this last case, *Prenolepis imparis* takes advantage on its high tolerance to cold conditions, beginning its activity and increasing its numbers before the Argentine ant starts its activity. As in our study place, stressful conditions allow some native ants to compete successfully against the Argentine ant.

According to our bait monopolization results *P. vistana* and *S. xyloni*, native species that mass recruited, had more success monopolizing baits, even if they were behaviorally subordinate species, like *P. vistana*. One explanation for this apparent importance of mass recruitment over behavioral dominance could be that behaviorally subordinate species that mass recruit usually arrive first at the bait and recruit in high numbers (Cerdá, personal communications). If the number of individuals of the subordinate species is too large, when behaviorally dominant species arrive they may not start a confrontation and the bait would remain monopolized by this species (Fellers 1987; Cerdá et al. 2013). In other regions, species with similar characteristics (behaviorally subordinate but with mass recruitment) have been observed co-occurring with the Argentine ant. This is the case of *Monomorium* genus, species that even if they are not behaviorally dominant, possess potent venom that use in encounters with other ant species and may dissuade them from attacks (Andersen et al. 1991). For example, *M. sydneyense* Forel, 1902 in Australia (Rowles & O'Dowd 2009), or *M. ergatogyna* Wheeler 1904, in Northern California (Holway et al. 2002a).

It is common to find behaviorally subordinate without mass recruitment species co-occurring with the Argentine ant, as we showed in the literature review. Some of them can use a marked submissive behavior in face of the

Argentine ant, like *Plagiolepis pygmaea* (Latreille, 1798) (Gomez & Espadaler 2006; Abril & Gómez 2009). Other behaviorally subordinate species that do not mass recruit might also co-occur with the Argentine ant given its low tolerance to extreme temperatures, like *Cataglyphis floricola* Tinaut, 1993 and its sister species *C. tartessica* Amor & Ortega, 2014, or *Aphaenogaster senilis* Mayr, 1853 in Spain (Angulo et al. 2007, 2011; Amor & Ortega 2014). In the case of *D. insanus* in our experiment, its activity at bait monopolization was higher than the behaviorally dominant *F. moki*, and could be due to their differences at recruiting; although both are not mass recruiters, *D. insanus* showed group recruitment while *F. moki* did not.

In other arthropods, the differential role of life history traits in competitive interactions among invaders or with native species can predict their co-occurrence or invasion success (Hogg & Danee 2011; Perderau et al. 2011; Gidoin et al. 2015). Our results showed how native species could offer less or more competition to the Argentine ant in harsh abiotic conditions, and we suggested that the Argentine ant's activity at baits was most strongly reduced when interacting with the native species that exhibited mass recruitment. In order to predict the competitive ability of native species against the Argentine ant, further studies are needed that focus on native species' behavioral traits and that use larger numbers of native species. The study of behavioral traits may allow us then to predict the likelihood of native ant communities interfering in the invasion of the Argentine ant in areas where the invader could arrive but abiotic conditions are not optimal for its invasion.

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SUPPLEMENTARY MATERIAL

List of Supporting Information items:

Table S1. Selection of the dependent variables' distribution models in the short-term removal experiment.

	Distribution model	Argentine		Natives	
		AIC	df	AIC	df
A	**Discovery time**				
	Poisson	NC	NC	NC	NC
	Negative Binomial	**0.0**	**11**	**0.0**	**9**
	Quasipoisson	2.6	11	12.1	9
B	**Recruitment time**				
	Poisson	153.3	11	NC	NC
	Negative binomial	**0.0**	**11**	**0.0**	**11**
	Quasipoisson	22.6	11	11.8	11
C	**Max ants**				
	Poisson	NC	NC	498.2	11
	Negative binomial	**0.0**	**11**	**0.0**	**11**
	Quasipoisson	21.4	11	3.7	11
D	**Accumulated # ants**				
	Poisson	NC	NC	NC	NC
	Negative binomial	69.8	10	63.4	10
	Quasipoisson	**0.0**	**10**	**0.0**	**10**

Table S2. Results of the pair-wise post-hoc comparisons with Bonferroni corrections to analyze if there were differences among the number of baits monopolized per species.

Pair of species	Adjusted χ^2 p-value
SX – LH	1.000
SX – PV	0.590
SX – DI	**< 0.001**
SX – FM	**< 0.001**
LH – PV	0.799
LH – DI	**< 0.001**
LH – FM	**< 0.001**
PV – DI	0.121
PV – FM	**< 0.001**
DI – FM	0.076

Table S3. Effects of different parameters on bait monopolization.

Species	**Day**			**Time of day**			**Temperature**		
	χ^2	p	N	χ^2	p	N	χ^2	p	N
L. humile	**20.36**	**<0.001**	**117**	2.24	0.135	117	**13.45**	**<0.001**	**117**
D. insanus	1.50	0.224	117	0.02	0.886	117	**10.00**	**0.002**	**117**
P. vistana	**5.62**	**0.018**	**117**	**7.96**	**0.005**	**117**	0.92	0.338	117
S. xyloni	**30.21**	**<0.001**	**117**	1.24	0.266	117	0.99	0.320	117

Table S4. Results of the ANOVA tests to analyze differences when competing vs. when foraging alone for each species for the foraging activity parameters.

	Discovery time			**Recruitment time**			**Max. number of ants**		
	F	p	N	F	p	N	F	p	N
LH vs. SX	0.14	0.719	10	**6.09**	**0.039**	**10**	**10.49**	**0.012**	**10**
LH vs. PV	0.09	0.770	10	1.57	0.245	10	0.20	0.664	10
LH vs. FM	0.01	0.920	8	1.54	0.262	8	**15.52**	**0.008**	**8**
LH vs. DI	1.40	0.271	10	0.44	0.528	10	0.06	0.814	10
SX vs. LH	1.82	0.214	10	2.91	0.126	10	2.80	0.133	10
PV vs. LH	3.09	0.117	10	4.66	0.063	10	3.95	0.082	10
FM vs. LH	4.87	0.069	8	-2.31	1	8	**8.00**	**0.030**	**8**
DI vs. LH	2.44	0.157	10	**6.15**	**0.038**	**10**	3.91	0.083	10

Table S5. Co-occurrence of native ant species with the invasive Argentine ant. Species could co-occur with the Argentine ant or be sensitive to displacement by the Argentine ant (Y, N in the “Co.occ” column respectively), based in the references in the column “Ref1” listed below. Life history traits were obtained from references in the column “Ref2” listed below, for behavioral dominance (dominant or subordinate ants, dom or sub in the column “Behav” respectively) and recruitment types (mass recruiters, group recruiters or solitary foragers, 1, 0.5 or 0 in the column “Recruit” respectively). Note that in the rest of the article, group recruiters and solitary foragers are considered as no mass recruiters. In the “Origin” column indicates the searching method (For, Gant or Biblio, for FORMIS, the Global Ant Database and the literature, see methods). The location and continent were the information was obtained is described in the columns “Location” and “Cont” respectively. Potential reasons for co-occurrence are showed in column “Reas” when are known. They include thermal niche, T; small worker body size, B; hypogeic, H; competitive superiority, C; submissive or inconspicuous behavior, S; or unknown, ?.

ANT SPECIES	Co.occ	Behav	Recruit	Origin	Location	Cont	Ref1	Ref2	Reas
Aphaenogaster gemella	N	sub	0.5	Biblio	Balearic I., Spain	Eu	15	33	
Aphaenogaster senilis	N	sub	0.5	For/Bib	Doñana, Spain	Eu	2, 10	1, 2, 29	
Anoplolepis custodiens	N	sub	0.5	Formis	W. Cape, S. Africa	Af	36	25	
*Bothriomyrmex meridionalis**	Y	sub		Biblio	Portugal	Eu	55	29, 33	H
Camponotus pilicornis	N	dom	0.5	Biblio	Doñana, Spain	Eu	10	5, 29	
Camponotus semitestaceus	N	dom		Biblio	California, USA	Am	23, 25, 44	29	
Cardiocondyla mauritanica	Y	sub	0.5	Biblio	Spain/USA	Eu+Am	15, 54	2, 29	?
Cardiocondyla nuda	Y	sub		Gant	Australia	Oc	20, 29	29, 32	S
Cataglyphis floricola	Y	sub	0	For/Bib	Doñana, Spain	Eu	10	1, 2, 29	T
Crematogaster auberti	N	dom	0.5	Biblio	Doñana, Spain	Eu	10	1, 2, 29	
Crematogaster californica	Y	dom	1	Biblio	California, USA	Am	60	29	?
Crematogaster peringueyi	N	dom	1	Biblio	W. Cape, S. Africa	Af	33,34	12	
Crematogaster scutellaris	Y	dom	1	For/Bib	Portugal	Eu	7, 55, 60	2, 29	C
Dorymyrmex insanus	Y	sub	0.5	Biblio	California, USA	Am	34, 48, 60	29, 30, 31	?
Forelius mccooki	Y	dom	1	Biblio	California, USA	Am	21	9, 20, 29	?
Formica francoeuri	N	dom		Gant			14, 23, 62	29	
Formica moki	Y	dom	0	Biblio	California, USA	Am	43, 60	29, 30, 31	?
Heteroponera imbellis	Y	sub		Biblio	Australia	Oc	42		B, H
Iridomyrmex bicknelli	N	sub	1	For/Gant	Victoria, Australia	Oc	41, 63	16,17	
Iridomyrmex rufoniger	Y	dom	1	For/Bib	Adelaide, Australia	Oc	52	26	C
Lasius grandis	Y	dom	1	For/Bib	Portugal	Eu	57	2, 29	C
Lasius japonicus	N	dom	1	Biblio	Hiroshima, Japan	As	39, 50	23	
Lepisiota capensis	N	dom	1	For/Bib	W. Cape, S. Africa	Af	36, 45	14	

Meranoplus peringueyi	Y	dom		Biblio	W. Cape, S. Africa	Af	28,36	10	S
Messor capensis	N		0	Biblio	W. Cape, S. Africa	Af	45	7, 11	
Monomorium antarcticum	Y		1	Formis	New Zeland	Oc	19	8	
Monomorium antipodum	Y	sub		Biblio	New Zeland	Oc	56	27	C
Monomorium sydneyense	Y	sub	1	Gant	Australia/New Zeland	Oc	42, 58, 61	11, 17, 29	C
Ocymyrmex barbiger	Y		0.5	Biblio	W. Cape, S. Africa	Af	45	11	T
Ocymyrmex cilliei	Y	sub		Biblio	W. Cape, S. Africa	Af	28	28	?
Pachycondyla chinensis	Y	sub	0	Biblio	North Carolina, USA	Am	13, 47	11, 22, 29	T
Pheidole capensis	N	sub	0,5	For/Bib	W. Cape, S. Africa	Af	36, 45	14	
Pheidole indica	N	dom	1	Biblio	Hiroshima, Japan	As	18, 39, 51	19	
Pheidole megacephala	N	dom	1	For/Gant	Australia/S. Africa	Oc+Af	20, 37	19, 29	C
Pheidole noda	N	dom	1	Biblio	Hiroshima, Japan	As	39, 51	19, 23, 29	
Pheidole pallidula	Y	dom	1	Gant	Portugal/Spain	Eu	16, 55	1, 2, 29	C
Pheidole vistana	Y	sub	1	For/Gant	California, USA	Am	30, 31, 60	29, 30, 31	?
Plagiolepis pygmaea	Y	sub	0.5	Gant	Spain	Eu	1, 15, 16, 38, 40	1, 2, 29	S
Pogonomyrmex californicus	N	sub	0	Biblio	California, USA	Am	11, 48, 60	8, 29	
Pogonomyrmex subnitidus	N	sub	1	Biblio	California, USA	Am	9, 48, 59	29	
Prenolepis imparis	Y	dom	1	Gant	California, USA	Am	12, 17, 22, 25, 34, 43, 44, 46, 48, 53	21, 29	T
Prenolepis sakurae	Y	sub		Biblio	Hiroshima, Japan	As	27, 35, 50	23	B
Prolasius advenus	Y		1	Formis	New Zeland	Oc	19	3, 8	T
Rhytidoponera victoriae	N	sub		Biblio	Australia	Oc	42	18	
Solenopsis invicta	N	dom	1	Gant			4, 32	24, 29	
Solenopsis molesta	Y	dom	1	Gant	California, USA	Am	14, 25, 34, 48, 49	29	B, H
Solenopsis xyloni	Y	dom	1	For/Gant	California, USA	Am	30, 31, 60	29, 30, 31	?
Tapinoma nigerrimum	Y	dom	1	For/Bib	France/Portugal	Eu	5, 6, 55	1, 2	C
Temnothorax andrei	Y	sub		Biblio	California, USA	Am	14, 24, 25, 34, 48, 49	13, 29	H, B
Temnothorax aveli	N	sub	0.5	Biblio	Doñana, Spain	Eu	10	2, 29	
Temnothorax wollastoni	N	sub	0	Biblio	Portugal	Eu	57	33	

Tetramorium caespitum forte	Y	dom	1	Biblio	Portugal	Eu	55	2	C
*Tetramorium sericeiventre***	Y	sub	0.5	Biblio	W. Cape, S. Africa	Af	36, 45	14	T
Tetramorium simillimum	Y	sub		Gant	Australia	Oc	19	6, 29	S
Veromessor andrei	N	sub	1	Biblio	California, USA	Am	25, 60	29, 30, 31	
Wasmannia auropunctata	Y	dom	1	Gant	New Caledonia	Oc	3	4, 15, 29	C

* *Bothriomyrmex meridionalis* / no valid: *Tapinoma meridionalis /meridionale*

** *Tetramorium sericeiventre* / no valid: *T. quadrispinosum*

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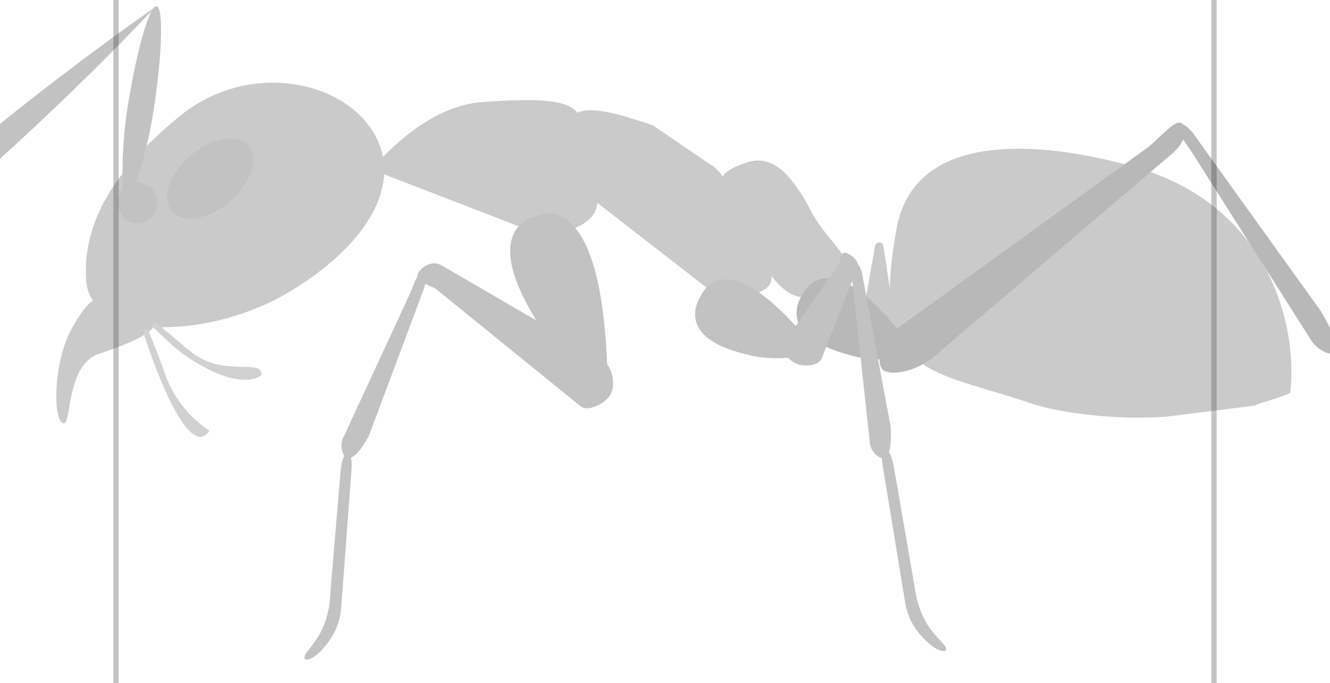
30. Personal Observations

31. Expert: David Holway

32. Expert: Xavier Arnan

GENERAL DISCUSSION AND CONCLUSIONS



1. GENERAL DISCUSSION

Invasive species are a major threat to native ecosystems, but their establishment and their success at spreading may be over-represented. There are many studies focusing on the life history traits that make species successful invaders, or on the impacts they have on the invaded ecosystems. For example, the Argentine ant, *L. humile,* has spread all over the world in Mediterranean-climate areas, and it is considered a successful invader due to its high rate of spread and its displacement of most native ants. Here, we have explored the failure and unsuccessful invasions of the invasive Argentine ant. We focus on what factors could be affecting several aspects of the Argentine ant invasion: its spread, success, and performance.

My results show that in some of the study areas the Argentine ant has spread over the years, but in some other areas populations have not been able to dominate the ant community, even after several decades of invasion. Environment can play a role in the invasion process, but the abiotic conditions by themselves do not seem to determine the success of the invasion in some areas. Native ant communities of invaded ecosystems can have a role, and complement the effect of environment and abiotic conditions, helping to determine the degree of success of the invasion. Although richness, diversity or structure of native ant communities may not be providing biotic resistance, there are traits related to interspecific competition that could be altering the outcomes in certain situations, like when abiotic conditions are not completely suitable for the invader. With respect to the spread of the Argentine ant invasion, I corroborate the importance of humans in the spread at a regional scale (e.g. within Mediterranean islands) and also at local scale, (i.e. at Doñana National Park, Spain). I also show that transport by scavenger raptors is possible at the local scale. To conclude, this dissertation highlights the importance of studying which factors of native ecosystems can modulate the degree of invasion, taking into account both biotic and abiotic factors and their interaction in the long-term.

1.1. Invasion success and spread of the Argentine ant

The Tens Rule states that of all introduced species, only 10% become established and that only another 10% of those become invasive (Williamson and Fitter 1996). This rule is the result of several causes of invasion failure at different stages of the invasion process (transport, introduction, establishment, spread; Blackburn et al. 2011). However, there are authors that disagree with this rule, arguing that it underestimates the numbers of successful establishments for at least certain taxa and diminishes perception of the threat invasive species represent (Lapointe et al. 2012; Jaric & Cvijanovic 2012). It is difficult to estimate the accuracy of successful establishments due to the lack of data on failed invasions, (Zenni & Nuñez 2013) and thus filling these gaps is essential for adequate management of invasive species.

In my dissertation, I explored failed invasions of the Argentine ant in terms of not being able to succeed completely at invading. I used published data of invasion status both locally and regionally, but more importantly, I obtained information for the long term by sampling invaded and uninvaded areas that were studied decades ago. By studying localities in which I knew the past invasion status, I could observe the success of the Argentine ant in spreading and in displacing native ant communities. Surprisingly, at the local scale in Doñana National Park (Spain), the Argentine ant has not only been incapable of expanding to a larger number of cork oaks in the last 16 years, but neither was it able to dominate in high numbers its already colonized range. Moreover, 8 of the 105 trees invaded in the past were recolonized by native ant species in 2016. This retraction of the invasion has been observed before in other Mediterranean-climate localities, such as Northern California, by Menke et al. (2018). Other studies have shown temporary retraction of Argentine ant range during cooler seasons, when they regroup the colonies looking for locations with suitable temperature and humidity, to spread again during the summer (Heller & Gordon 2006; Abril et al. 2008; Heller et al. 2008; Díaz et al. 2013; Burford et al. 2018). For both temporary and permanent retractions of Argentine ant populations, some authors suggest that variations in relative abundance of the Argentine ant are more likely caused by site-specific factors than by time since invasion, and I agree this might be the case in Doñana. In fact, invasion at Doñana is patchy, with Argentine ants occupying the humid and cool cork oaks while avoiding the dry scrubland (Angulo et al. 2011). At unsuccessfully invaded plots within the invaded area, or on the borders of this patchy invasion, the Argentine ant is in constant competition with native ants; small changes in abiotic conditions, like a drier season or a drier microhabitat, could provide native species with the advantage they need to recolonize terrain.

Similarly, at the local scale in the beaches of the studied Mediterranean islands, the Argentine ant co-occurred with native species in almost half of the invaded beaches in Ibiza and Formentera, and in one-third of the beaches in Corsica. Moreover, in at least 4 of these beaches invader and native ants have been co-occurring for over a decade.

According to Suarez et al. (2001), the maximum annual rate of spread of the Argentine ant invasion is 154 ±21 m (range 15 - 275 m). Taking into account that the maximum length of these unsuccessfully invaded beaches was 1032 m, and in Doñana the maximum distance among invaded and uninvaded trees was 329 m in 2016, the Argentine ant could have been able to colonize the entire beaches and the entire invaded area at Doñana over these years. These spreads could have occurred if both abiotic and biotic conditions had been suitable for the Argentine ant invasion. The long-term temporal scale used in this dissertation has shown that invasion by the Argentine ant is not always successful in the long-term and that specific local factors, like biotic and abiotic conditions of the recipient communities, are essential to understanding its success or long-term failure.

Some populations of invasive species oscillate substantially over time and end up collapsing for no apparent reason (Simberloff & Gibbons 2004; Lester & Gruver 2016). The most noticeable case among ants is *Anoplolepis gracilipes*, a species whose populations tend to fluctuate both spatially and temporally. In Australia its populations have declined greatly over time or even disappeared completely without human intervention (Cooling & Hoffmann 2015). This mechanism can also operate on Argentine ant populations, as observed in New Zealand, California, and Madeira (Lester & Gruver 2016). Many factors, both direct and indirect, could be causing the decline of populations, but they are still unknown. One of the causes that has been hypothesized to initiate populations' collapse is over-exploitation of key resources (Lester & Gruver 2016). In those habitats where the Argentine ant has not been able to completely succeed and is present at lower abundance, high levels of competition with the native ant community for these resources could trigger a decrease in Argentine ant abundance, resulting in local extinction. This situation would be aggravated if populations are located in a isolated site like an island, where the recovery would be more difficult and extinction more likely (Simberloff & Gibbons 2004). Continuous monitoring of populations of the Argentine ant, especially those that are unsuccessful at invading, are of extreme importance to the future management of this species. If we do not keep track of populations we will not have accurate data on how long the Argentine ant can maintain unsuccessful invasions, why they are unsuccessful, how long it could take populations to collapse, how often collapses happen, and what factors lead to collapse. According to Simberloff and Gibbons (2004) data quantifying these decreases are very rare because researchers' interest in the invasion is reduced once the decline has taken place. Here, I show that the Argentine ant maintained unsuccessful invasions for decades (24 years for Doñana, 12 years for Ibiza and Formentera, and over a decade in Dawson Los Monos Canyon, California, USA). A similar pattern has been observed in a regional long-term study in California (USA), where the Argentine ant has been maintaining populations for over three decades without being able to reach high abundances nor displacing 40% of the native ant community (Menke et al. 2018). Argentine ant invasion in Madeira (Portugal) has proved not to be successful although the ant has been present in the area for more than a century and a half (Wetterer et al. 2006). I draw attention to the need to fill these gaps in our knowledge of biological invasions, in order to avoid bias and to help design effective management.

1.2. The role of environment on Argentine ant spread

The successful spread of the Argentine ant over Ibiza, Formentera and Corsica, the lack of success in some of the beaches of the Balearic Islands over time, and the lack of expansion in Doñana Biological Reserve or in the Natural Reserve of Dawson in Southern California, could be modulated by differences in abiotic conditions such as temperature or moisture. Previous studies have shown that these abiotic factors can restrict Argentine ant abundance. For example, abundance is higher in areas with higher vegetation cover than in scrublands (Way et al. 1997; Holway 2005; Menke et al. 2007; Rowles & O'Dowd 2007; Angulo et al. 2011; Roura-Pascual et al. 2011). Nevertheless, our results showed that this generalization may not hold when accounting for site-specific conditions. For example, larger trees in Doñana, which provide favorable moisture and temperature conditions, were not more likely to be invaded than smaller ones.

The vegetation cover around the trees did not facilitate invasion either. This result agreed with the study of Carpintero et al. (2005) on Doñana cork oaks, which found that the invasion was independent of tree size. Indeed, the invaded beaches in Ibiza and Formentera were the ones with less dense vegetation. This seems to indicate that abiotic conditions by themselves are not preventing the spread of the Argentine ant (although they can shape its distribution), and that others factors are determining the success of those invasions. Several studies agree with this idea, emphasizing the importance of fine-scale changes in physical environment to community invasibility, and the need to study the interactions between the biotic and abiotic environment (Menke & Holway 2006; Menke et al. 2007; Ricciardi et al. 2013; Gao & Reitz 2017).

1.1. The role of native ant communities in the success of the Argentine ant

This dissertation corroborates previous studies that did not support the biotic resistance hypothesis of the Argentine ant in terms of richness or diversity (e. g. in Holway 1998; Rowles & O'Dowd 2007). Although I did not find significant results in ant community structure either, I observed a pattern in community structure regarding likeliness of invasion and its success. In Chapter II at local scale in Doñana, native ant community structure of those trees that were invaded was narrower than in trees that remained uninvaded. In Chapter III at regional scale, this pattern was repeated, but the narrower structure belonged to the control plots of unsuccessfully invaded localities relative to the control plots of successfully invaded localities. Taken together, results at different scales seem to support the hypothesis that the ability to resist invasion depends more heavily on the structure of the native ant community than on number of species or the diversity of the ant community (Crawley et al. 1999; Emery & Gross 2006; Dzialowski 2010; Henriksson et al. 2015).

I explored this possibility by testing if the identity of resisting species corresponds to specific traits related to interspecific competition, such as behavioral dominance or recruitment strategy. Results in Chapter II at the local scale in Doñana and Chapter III at the regional scale were in disagreement with this hypothesis: higher presence of behaviorally dominant species or mass recruiters in the original native community did not prevent the invasion of the

Argentine ant, or did not influence the degree of its success. However, using behavioral experiments in California (Chapter IV), I tested how Argentine ant recruitment to baits was differentially affected by competition with species holding different combination of these traits. Although statistical analysis did not indicate that changes in Argentine ant recruitment were caused by these two traits (this might be derived from the small number of species analyzed and further experiments with more species are needed in order to draw stronger conclusions), the literature review supported my hypothesis. I showed that ant species that were described as co-occurring with the Argentine ant were in higher proportion both behaviorally dominant and mass recruiters. This has been the case with species like *Crematogaster scutellaris*, *Lasius grandis, Pheidole pallidula, Iridomyrmex rufoniger,* or *Tetramorium forte* (Way et al. 1997; Paiva et al. 1998; Walters & Mackay 2005; Wetterer et al. 2006). In Corsica, one of my study areas, *Tapinoma nigerrimum* s.l. seems to be limiting the Argentine ant spread and success (Blight et al. 2010a). When the Argentine ant arrives to a new environment and establishes successfully, it usually displaces these ecologically similar species first (Carpintero et al. 2003; Mothapo & Wossler 2014). However, these same species can be the ones that, in combination with other factors like stressful environments for the invader, present biotic resistance to the invasion through competition. Menke et al. (2007) illustrated that the presence of native ants can slow the invasion of Argentine ants under certain environmental conditions. If abiotic conditions can help the native ant community slow down the invasion, the presence of certain key species might prevent it completely.

The presence of species with higher competitive abilities would also interfere with the ability of the Argentine ant to acquire the resources it needs for a successful invasion. I hypothesized that the presence of an empty trophic niche in certain areas could fuel the success of the invasion. In the successfully invaded areas I explored in Chapter III there was no evidence that the success of invasion was explained by niche availability; the native community exploited baits with similar effectiveness in areas successfully and unsuccessfully invaded. Nevertheless, Angulo et al. (2011) showed that the Argentine ant removes carcasses in invaded habitats faster than native species do in uninvaded habitats, so even if the niche is not available, the Argentine ant can get control of it and exploit it more efficiently. To the best of my knowledge, this dissertation is the only study analyzing the effect of niche availability on the success of Argentine ant invasion. However, several studies have analyzed the relationship between the availability of different type of resources and invasion. The Argentine ant needs high levels of protein in the establishment stage, but increased income of carbohydrates increases its likelihood of establishment (Shik & Silverman 2013; Shik et al. 2014) and its abundance (Rowles & Silverman 2009). However, Phair (2004) suggested that at this stage it is very likely that colonies are too small to be able to establish successful mutualistic interactions. In addition, previous results on the Argentine ant have shown how it decreases its level of aggression when sucrose is scarce (Grover et al. 2007). In pine forests like our study areas, aphids constitute an important food resource for ant species (Mooney & Tillberg 2005), so sugar would not be a scarce resource for Argentine ants there.

Differences in aggression could be due to other factors related to behavioral plasticity. The Argentine ant is known to display behavioral plasticity in interspecific confrontations. Blight et al. (2010a) observed that the Argentine ant employed submissive behaviors like thanatopsis (death-feigning) in confrontations against the behaviorally dominant species *Tapinoma nigerrimum*, a strategy that is more commonly used by submissive species like *Plagiolepis pygmaea* (Abril & Gomez 2009). This behavior may enable the invader to co-occur with dominant species when it is not able to displace them. Abril and Gomez (2009) also compared Argentine ant aggression between populations that either co-occurred or did not co-occur with a native species. They did not find differences in levels of aggressiveness of the Argentine ant according to its co-occurrence with native species. This result suggested that co-occurrence is not caused by an habituation process, and at the same time it supports the hypothesis of the frequent co-occurrence between Argentine ants and *P. pygmaea* due to *P. pygmaea*'s submissive behavior. Menke et al. (2018) tested variation in aggressiveness according to time since invasion, using historical data and aggression tests, and they found that aggression levels of the Argentine ants were time independent.

1.4. The expansion of the different supercolonies

The ability to form supercolonies is one of the most characteristic traits of the Argentine ant, which facilitates its worldwide success (Passera 1994). Although uncommon, supercolonies have been found in contact with each other and with no aggression between them in some cases. For example, in southeastern France ants from the Corsican and the Main supercolonies tolerate each other in a border zone (Berville et al. 2013). In our study of the three European supercolonies in Mediterranean islands (Chapter I) we observed that supercolonies in the three islands seem to be excluding each other, similar to other invaded areas maintaining several different supercolonies (Thomas et al. 2006; Sunamura et al. 2009). This exclusion is derived from the high intra-specific aggression that supercolonies display (Buczkowski et al. 2004; Jaquiéry et al. 2005; Thomas et al. 2006). According to this, it is likely that the expansion of different supercolonies would be determined by the history of the invasion; the first supercolony that arrives to a new place would likely exclude other supercolonies that arrive later. This has been the case in Ibiza and Corsica in my study, where the Main European supercolony was the first to establish and is the most widespread in the islands. However, I found in Formentera that although the Catalonian supercolony arrived first to the island, the Main supercolony colonized more sites one decade after. This may be caused by differences among supercolonies in behavioral traits that are correlated with ecological success; the Main supercolony was more active at exploring new environments, and it is more aggressive and detects food resources more quickly, which facilitates its spatial expansion over the Catalonian (Blight et al. 2017). It would be interesting to track first introductions of the Argentine ant worldwide in regions where several supercolonies occur, to test the hypothesis that the first supercolony arriving is the one that spreads the most. Regions such as California (Tsutsui et al. 2003) and Hawaii (van Wilgenburg et al. 2010) in USA, southern France (Blight et al. 2010b), Japan (Sunamura et al. 2009), South Africa (van Wilgenburg et al. 2010), and Spain (Giraud et al. 2002) could improve our knowledge of this field.

1.5. Future perspectives

This dissertation focuses on the invasibility of ecosystems and whether and how they can modulate Argentine ant invasion success. Across the four Chapters, the variability in the results obtained for biotic, abiotic, and environmental factors indicates that these factors cannot be studied independently and should be considered altogether to understand how their interactions can affect the

Argentine ant's invasion performance. This opens a door to several future lines of inquiry in order to deepen our understanding of how these factors relate.

First of all, it is important to draw attention to the lack of information about failed invasions. Analyzing only successful invasions provides a bias in researchers' assumptions (Nuñez & Medley 2011; Rodriguez-Cabal et al. 2013; Zenni & Nuñez 2013). There is difficulty in reporting failed introductions. However, with the Argentine ant I was able to characterize invasion success according to two characteristics: the invader abundance level, and its impacts on the ant community (i.e. native ant species displacement). In my dissertation I have not considered failed introductions of the Argentine ant, which would provide us information about factors affecting the first steps of the invasion process of ant propagules (transport, introduction, establishment; Blackburn et al. 2011), as well as abiotic factors determining the Argentine ant's distribution outside its native range. However, there is already a body of knowledge on the importance of propagule size and the castes within this propagule (see e.g. Hee et al. 2000; Sagata & Lester 2009; Luque et al. 2013; Angulo et al. 2018), as well as on the abiotic factors that are required for Argentine ant invasion (Holway 1998; Human et al. 1998; Suarez et al. 2001; Holway et al. 2002; Roura-Pascual et al. 2011).

The characterization of successful and unsuccessful Argentine ant invasions allowed us to study what biotic or abiotic factors could prevent the spread stage of the invasion once the barriers of transportation, introduction, and establishment are overcome. However, in my dissertation abiotic factors were less explored than the biotic ones, and it would be very interesting to extend this inquiry in future projects. For example, climatic variables such as temperature and moisture could have been measured or obtained to correlate with invasion success at beaches of Mediterranean islands (Chapter I), at the local scale in Doñana (Chapter II), or at the regional scale (Chapter III).

To my knowledge, this is the first study that focuses on the unsuccessful invasion of the Argentine ant. However, there are some reports of unsuccessful invasions in different regions, such as in Australia (Thomas & Holway 2005) or Madeira, Portugal (Wetterer et al. 2006). A way forward to understand what limits the spread stage of the Argentine ant could be a global comparative analysis. For example, long-term analyses to determine invasion success and experimental and observational methods could be used to measure abiotic and biotic factors in paired plots (e.g. invaded and control plots, successful and unsuccessful plots). This approach would allow a broader-scale exploration of my hypotheses, and would complement my study by including a set of abiotic factors that could be interacting with the biotic ones to interfere in the success of the invasion.

In this dissertation, I have analyzed the progress of invasion in different localities at different spatial scales and over several decades. Continuous monitoring of these populations is necessary to detect possible fluctuations, retractions, and even collapse (e.g. Sanders et al. 2001; Cooling et al. 2012; Menke et al. 2018). In my study, long-term monitoring has been essential to show that Argentine ant invasions may be unsuccessful, and that these situations are more frequent than we expected. I propose that worldwide long-term monitoring could be complemented by periodic sampling of the native ant communities (for example, with pitfall traps), and abiotic (local temperature and moisture) and environmental variables (habitat, soil, vegetation cover). Thus, we could obtain a database that could reflect not only how the Argentine ant invasion evolves in each location over time, but also to understand how the variables sampled could affect the invasion globally.

It is still unclear how native ant resistance against the Argentine ant depends on traits related to interspecific competition. The result of my dissertation suggest that these traits may be interacting with other (abiotic) factors to promote native species co-occurrence with the Argentine ant, and thus providing biotic resistance through interspecific competition. Having a better understanding of how native species' traits may interfere with Argentine ant performance would allow researchers to include this factor in predictive models of predictive models of suitable habitat. Models predicting introduction of invasive species based on climatic factors usually do not include biotic interactions (Nuñez & Medley 2011). Previous studies have demonstrated the failure of models to predict spread of alien species (Broennimann et al. 2007; Fitzpatrick et al. 2007). Designing predictive models that encompass both climatic suitability for the Argentine ant and the invasibility of these areas in terms of environmental and biotic factors would allow us to more effectively manage vulnerable areas.

Altogether, my results indicate that Argentine ant invasion is not always successful and that invasion success relies on both biotic and abiotic factors of the recipient ecosystem. These factors do not act independently and are not static in time, producing variability in invasive species populations. Therefore, periodic population tracking and long-term monitoring are necessary to understand the complexity of the dynamics of biological invasions.

2. CONCLUSIONS

1) The Argentine ant (*Linepithema humile*) was not always successful at invading. In areas unsuccessfully invaded, Argentine ant nests did not completely saturate the invaded area, which allowed native species to co-occur with it. Unsuccessful invasions can remain in this status for more than a decade, suggesting that invasion success is not necessarily time-dependent. Long-term monitoring of the Argentine ant populations allows detection of unsuccessful invasions, and also reveals invasion fluctuations and retractions, like the one observed in some cork oaks at Doñana National Park.

2) The biotic resistance hypothesis, in terms of native ant diversity, richness, or community structure, did not apply alone to protect native ant communities against the Argentine ant invasion, but could be acting in combination with site-specific abiotic and environmental factors to prevent the success of the Argentine ant. Success of the invasion was not determined by the availability of an empty trophic niche.

3) Traits involved in interspecific competition, such as foraging recruitment strategy or behavioral dominance, may play a role in the success of the invasion. The proportion of species possessing behavioral dominance and a mass recruitment strategy did not influence the likeliness of invasion or the success of invasion in our study sites. However, species presenting such traits could have greater chances of resisting the invasion through competition, as they co-occur more frequently with the Argentine ant in unsuccessfully invaded areas of different parts of the world, and are capable of reducing foraging performance of the Argentine ant in bait competition experiments.

4) The Argentine ant showed increased aggressiveness against native ants in areas that were unsuccessfully invaded, where the Argentine ant was in constant competition with native species, relative to areas successfully invaded where no native species occur.

5) The natural local spread of the Argentine ant is by budding, but it frequently uses vectors to colonize inaccessible areas. Beside humans, which seemed to be the main vector at local (e.g. at Doñana) and regional (e.g. beaches in Mediterranean islands) scales, scavenger raptors can transport Argentine ant propagules in carcasses at short and medium distances.

6) The three supercolonies (Main, Catalonian and Corsican) expanded along the past decades in the Balearic Islands and Corsica to beaches with more human presence and that were near already invaded beaches. The largest expansion was for the Main supercolony in Ibiza and Formentera. Interestingly, although the Main supercolony seemed to have been introduced first in most islands, this historical advantage did not result in greater expansion in Corsica, where the Corsican supercolony had greater expansion than the Main supercolony.

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ORIGINAL PAPER

Humans and scavenging raptors facilitate Argentine ant invasion in Doñana National Park: no counter-effect of biotic resistance

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Abstract Biotic resistance by native communities could have a role in the spread of invasive species. This seems to be the case in the invasion of the Argentine ant, *Linepithema humile,* but only when the environment is unfavorable for the survival of the invader. We studied the progress of Argentine ant invasion through favorable and unfavorable habitats of Doñana National Park across three temporal snapshots covering three decades (1992, 2000, 2016). We assessed biotic resistance of the native community using species richness, as well as dominance and community structure. We also explored the role of abiotic factors (quality of surrounding habitat and spatial variables) and of potential vectors of Argentine ant dispersal across unfavorable areas. We found no evidence of biotic resistance after examining native ant species richness, proportion of native dominant ants, or community structure. On the contrary, invasion proceeded from trees with higher ant species richness, probably because those trees are larger and provide more resources and better protection from aridity. Furthermore, we found evidence that the invasion of new trees across a matrix of unfavorable habitat could be influenced not only by humans, but also by scavenging avian predators, which could act as vectors of ant dispersal through transport of carrion also exploited by the ants. Such leapfrog expansion through mobile predators could represent an overlooked mechanism that would enrich our understanding of invasion dynamics and provide potential opportunities for management of invasive species.

Keywords Biotic resistance · Dominance hierarchies · Community structure · Dispersal by mobile predators · *Linepithema humile* · Raptors

Introduction

Biological invasions are a well-known worldwide threat to biodiversity, reducing or even replacing native species (Sax and Gaines 2003; Simberloff et al. 2013; Kumschick et al. 2015; Li et al. 2016). Different hypotheses can explain the success, spread, and impact of invaders, like propagule pressure, enemy release, biotic resistance or invasion meltdown hypothesis, among others (Catford et al. 2009; Ricciardi et al. 2013; Heger and Jeschke 2014; Jeschke

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2014). Ecosystems can be more resistant to invaders due to either abiotic factors, like environmental tolerance to temperature or moisture (Shea and Chesson 2002; Blackburn et al. 2011), or biotic factors, like food resources or natural predators and competitors (Shea and Chesson 2002; Kumschick et al. 2015). According to biotic resistance theory (Elton 1958), native communities with higher species richness should be less susceptible to invasion by exotic species than ecosystems with fewer component species. In this context, interspecific interactions can act as biotic barriers and prevent a new species from persisting in a highly competitive environment (Holway 1999; Parr 2008; Blight et al. 2014).

Ant communities are good models for testing the relative roles of biotic resistance in the spread of invasive species. Competition in ant communities is important in shaping community structure (Parr 2008; Arnan et al. 2012; Cerdá et al. 2013). Moreover, ants' unique life history traits (e.g. social structure, colony foundation, worker polymorphism) make the study of their movements, invasion dynamics and interactions with the native community interesting (Bertelsmeier et al. 2015, 2017). In addition, invasive ants are among the worst world invaders (Lowe et al. 2000), so understanding the role of biotic resistance is important for future management and prevention of its spread. Hölldobler and Wilson (1990) defined two main types of competition in ants. Exploitative competition refers to the ability of ants to rapidly detect a food resource and recruit a large amount of individuals to exploit it. Interference competition refers to the ability of ants to dominate a food resource through aggression. On the basis of these interactions, ant species have been classified mainly as dominant or subordinate (Vepsäläinen and Pisarski 1982; Savolainen et al. 1989; Arnan et al. 2012, 2017). Dominant species typically shape the species composition of ant assemblages (Cerdá et al. 2013), while subordinate species avoid contact with other species (Arnan et al. 2011). The latter strategy of avoidance allows subordinate species to coexist (Calcaterra et al. 2016). As a result, high rates of dominant species are not inversely proportional to species richness (Arnan et al. 2011).

The Argentine ant, *Linepithema humile,* is an example of a highly dominant ant (Human and Gordon 1996). It is also a highly invasive species, native to South America, which has successfully established around the globe (Lowe et al. 2000; Suarez et al. 2001). This ant possesses life history traits that make it an exceptionally good invader (Table 1; Arnan et al. 2012; 2017). It is unicolonial (individuals move freely among physically separate nests without showing any kind of intraspecific aggression; Holway et al. 1998; Giraud et al. 2002); its colonies are polygynous (elevated number of reproductive females; Hölldobler and Wilson 1977; Passera 1994) and polydomous (single colonies are divided in multiple physical nests; Pedersen et al. 2006; Heller et al. 2008); and it is highly aggressive toward other ant species through both physical and chemical attacks (Suarez et al. 1999; Welzel et al. 2018). When it colonizes a new area, the Argentine ant usually displaces the native ant community and produces a cascade of negative impacts on other taxa including arthropods, small vertebrates, and plants (Holway et al. 2002; Sanders et al. 2003; Rowles and O'Dowd 2009; Alvarez-Blanco et al. 2017). *Linepithema humile* has an overall generalized diet including insects, carrion or nectar (Holway et al. 2002; Angulo et al. 2011), and it has been shown that carbohydrate-rich diets provided by aphids favor its success (Rowles and Silverman 2009). The only aspect of this species' natural history that may constrain its colonization potential is the lack of winged queens, which can prevent dispersal across patches of unfavorable habitat (Way et al. 1997; Holway 1998).

Previous work has demonstrated that native ant communities do not show biotic resistance against Argentine ant invasion when abiotic conditions are favorable for the Argentine ant (Way et al. 1997; Holway 1998; Menke et al. 2007; Rowles and O'Dowd 2007; Roura-Pascual et al. 2011). On the other hand, some dominant native species are capable of resisting the invader Argentine ant. For example, species like *Lasius grandis*, *Tapinoma nigerrimum* or *Iridomyrmex rufoniger* can repel the invader when its propagule size is not adequate (Way et al. 1997; Walters and Mackay 2005; Wetterer et al. 2006; Blight et al. 2010), while some other species like *Crematogaster scutellaris, Pheidole pallidula* and *Tetramorium forte* can prevent its spread in habitats that are suboptimal for the Argentine ant (Way et al. 1997).

The Argentine ant expansion has mainly affected areas with a Mediterranean climate but can also be limited by abiotic factors, especially temperature and moisture (Carpintero and Reyes-López 2008; Roura-Pascual et al. 2009, 2011). Although localized

Table 1 Ant species present in the cork oak trees of the study area in 1992 and in 2016, and their life-history traits

Species	%		Life history traits				
	1992	2016	Dom	Queen	Nest	Forage	Diet
Linepithema humile	–	–	1	1	1	1	0, 1
Crematogaster scutellaris	76	84	1	0.5	0.5	1	0, 1
Camponotus lateralis	65	42	0	0	0	0.5	0
Colobopsis truncata	57	84	0	0	0	0	0
Lasius lasioides	49	11	1	0	0	0	0, 1
Camponotus cruentatus	41	0	1	0	0	0.5	0, 1
Cataglyphis spp.[a]	24	0	0	0	0	0	1
Temnothorax racovitzai	16	0	0	0	0	0.5	0, 1
Tapinoma cf. *nigerrimum*	11	5	1	1	1	1	0
Iberoformica subrufa	5	0	0	0	0	0.5	0, 1
Tetramorium forte	5	0	1	0	0	1	1, 0.5
Cardiocondyla batesii	3	0	0	0	0	0.5	0, 1
Plagiolepis schmitzii	3	0	0	1	0.5	0.5	0
Camponotus fallax	0	37	0	0	0	0.5	0
Lasius grandis	0	21	1	0	0	1	0, 1
Formica cunicularia	0	5	0	0	0.5	0.5	0, 1
Tapinoma erraticum	0	5	1	1	1	1	0

Species are sorted by their percentage occurrence (%) in the sampled trees in 1992 (N = 37 trees). Ant species in 2016 and their percentage of occurrence is also showed (N = 19 trees). Four life history traits related to competition in ant communities are given, following Arnan et al. (2012, 2017). Behavioral dominance (Dom): dominant (1) and subordinate (0). Number of queens (Queen): polygyny (1), monogyny (0) or both (0.5). Number of nests (Nest): polydomy (1), monodomy (0) or both (0.5). Foraging strategy (Forage): mass recruitment (1), group recruitment (0.5), individual foraging (0). Diet: liquid food (0), seeds (0.5) or insects (1). The Argentine ant is added as a comparison for these traits

[a] *Cataglyphis spp.* corresponds to two species, *C. floricola* and *C. tartessica*, the latter was described in 2014 and formerly was considered the orange form of *C. floricola* (Amor and Ortega 2014)

resistance offered by dominant species could be effective under certain environmental conditions, spread across abiotic obstacles could be favored by accidental facilitation, such as, human-driven jumps. For example, it has been shown that, at the global scale, long-distance Argentine ant dispersal is typically mediated by unintentional human transport (Suarez et al. 2001). Humans can also enable more local, short-distance jumps (Carpintero et al. 2005; Angulo et al. 2011), which are otherwise unlikely because of the wingless queens.

In this study, we aim to assess the relative roles of different factors affecting the spread of the Argentine ant at a local scale in a Mediterranean ecosystem with favorable habitats (cork oak trees) interspersed within an unfavorable scrubland matrix. We used a temporal approach, comparing snapshot-annual data of ant communities collected through three different decades (in 1992, 2000 and 2016) during the spread of the Argentine ant in the area. First, we tested whether the native species richness or the proportion of dominant ants in uninvaded cork oak trees in 1992 could be limiting the future invasion by Argentine ants. We hypothesized that trees with low ant species richness or low proportion of dominant ants would be invaded first, following the biotic resistance hypothesis. In order to further explore this hypothesis, we analyzed whether the community composition was related to the process of the invasion. We hypothesized that trees that were invaded first would have a different species composition compared to the ones that were not invaded. Alternatively, we explored the possibility that spread to new trees is facilitated by other factors. In the study area, transport of *L. humile* by humans and by scavenger raptors, which may take ant-infested carrion items to their nests, has been qualitatively suggested to influence the invasion of new trees (Carpintero et al. 2005; Angulo et al. 2011). In these cases, we would expect that the Argentine ant spread would be linked to roads and paths, or to the breeding sites of scavenging raptors.

Materials and methods

Study area

Sampling was conducted in Doñana Biological Reserve of Doñana National Park (37°1′N, 6°33′W), situated on the Southwest coast of Spain, in an open Mediterranean scrubland containing scattered pine forests (*Pinus pinea*) and isolated cork oak trees (*Quercus suber*) (Fig. 1). The study area is inhabited by more than 30 ant species, and the Argentine ant is the only invasive species (Carpintero et al. 2004). The first invasion focal point was the building of El Palacio de Doñana, the Reserve's field-research station with year-round human presence of some 3–50 people, where the Argentine ant was first detected in 1970 (Angulo et al. 2011). Due to its low tolerance of high temperatures and dry habitats, its range in natural habitats is mainly restricted to individual cork oaks or pine forests (Angulo et al. 2011). The first time the Argentine ant was seen in a natural area of Doñana National Park was in the 1980's in a cork oak in El Jaulón (Carpintero et al. 2005), a human construction that very likely constituted a second invasion focal point (Fig. 1). Thus, in our study, isolated cork oak

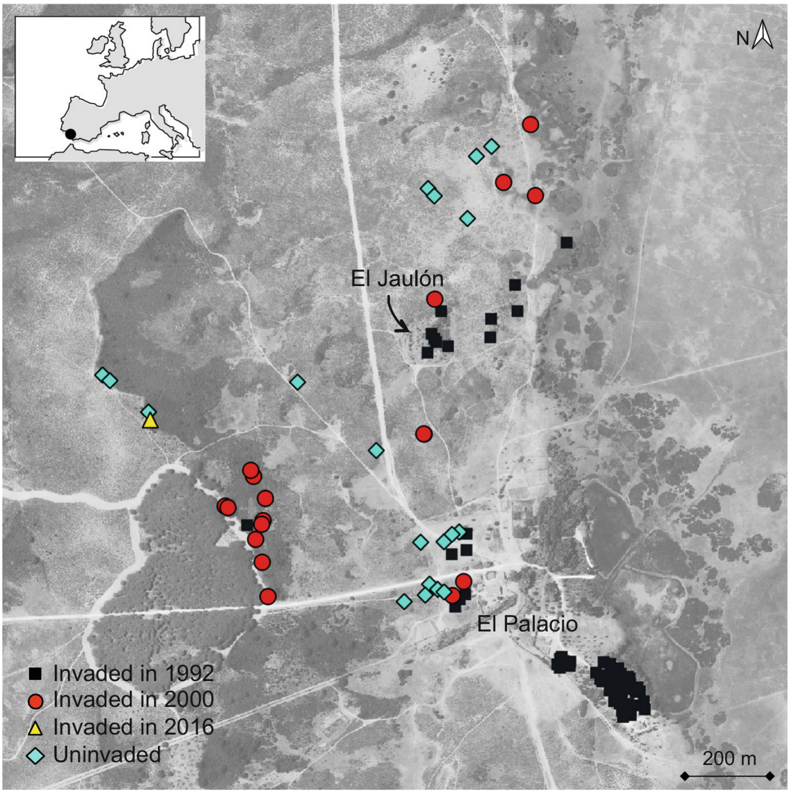

Fig. 1 Study area: the main focal points for the invasion of the Argentine ant, El Palacio and El Jaulón, are marked. The study area consists of a matrix of unfavorable dry Mediterranean scrubland containing scattered cork oak trees, which act as favorable habitat patches. Trees that were invaded in 1992 are marked by black squares, trees that were invaded in 2000 are marked by red circles, and trees that were still not invaded in 2000 are marked by light blue diamonds. The only tree invaded between 2000 and 2016 is marked by a yellow triangle

trees constitute our sampling units in order to follow the invasion progress of the Argentine ant.

Sampling the ant community

In June 2016 we repeated, using the same protocol, the sampling that Carpintero et al. (2005) performed in 1992 and 2000. They sampled the ant community at all the cork oaks within 1.5 km of the invasion focal point, El Palacio. At each tree, during 10 min, we looked for the different ant species, native or invasive, patrolling trunk and branches from 0.5 to 2 m high. We considered a tree invaded if the Argentine ant was present on it. In general, when the Argentine ant successfully invades a tree, it replaces all other native ant species, so its detection is very likely (Angulo et al. 2011). Samples of native ant species were collected and kept in 70% alcohol for subsequent identification by the authors in the laboratory.

From the 182 trees Carpintero et al. (2005) sampled, we excluded the ones that were not alive in 2016, and three trees that had two trunks one beside the other were considered only one tree. Thus, we get an initial sample size of 105 trees in 1992, of which only 38 were uninvaded at that time. Of these 38 trees, 18 had been invaded by 2000 and only a single additional tree had been invaded by 2016 (Fig. 1). Therefore, because expansion virtually halted after 2000 in the sampled area, here we focus on the process of invasion that occurred between 1992-2000, which covers a significant invasion expansion. Trees were categorized by their invasion status as "uninvaded" if they remained uninvaded in 2000 (N = 19) or "invaded" if they were invaded in 2000 (N = 18; Fig. 1), with a total number of 37 cork oaks.

Ant community structure

A matrix of presence-absence of each native ant species found in the 1992 survey was constructed for the 37 trees selected for the analysis. We compared the native ant community between trees that were subsequently invaded in 2000 or remained uninvaded by this year (invasion status). We performed a permutational multivariate analysis of variance (PERMANOVA) using the *adonis* function (package Vegan, Oksanen et al. 2016) in R (R Studio version 1.0.136, RStudio Team 2016).

To graphically represent differences in community structure by invasion status, we performed a non-metric multidimensional scaling test (NMDS), using the metaMSD function with 1000 iterations (package Vegan, Oksanen et al. 2016) in R (RStudio Team 2016). This analysis represents the original position of each community in multidimensional space. We chose the appropriate number of dimensions by examining the stress value (correct below 0.1). Trees were differentiated by their invasion status using the *ggplot* function (package ggplot2, Wickham 2009), which draws 95% confidence interval ellipses.

We were interested in whether the ant community of uninvaded trees had changed. We performed a second PERMANOVA and NMDS (as described before) to compare ant communities in 1992 and in 2016, using only trees that remained uninvaded in 2016 (N = 19).

Anthropogenic, biotic and abiotic variables affecting the invasion of new trees

In order to know which parameters affected the invasion of new trees since 1992, we performed a generalized lineal model using the *glm* function (package stats, R Core Team 2015). The dependent variable was the status of invasion (invaded or uninvaded in 2000) and we modeled it with a quasibinomial link distribution. We estimated the following parameters that we fitted to the model as independent variables:

(a) Ant species richness: number of native ant species for each cork oak tree in 1992. Species richness was added to the model in order to test the biotic resistance hypothesis, under the prediction that less species-rich trees would be liable to invasion.

(b) Behavioral dominance of native species: we separated the native species found in 1992 in each cork oak tree by their position in the behavioral dominance hierarchy, classing each as dominant or subordinate, following Arnan et al. (2012, 2017) (Table 1). We estimated the percentage of dominant species from the total number of native species present. Our prediction was that a lower proportion of dominant species would increase the likelihood of invasion.

(c) Spatial variables: The geographic coordinates (latitude and longitude) of each tree were added to the model to take into account the spatial arrangement of trees. We used a geographic information system (QGIS, version 2.18.14, QGIS 2013) to calculate distances from each sampled tree to three potential invasion sources: El Palacio, El Jaulón, and the nearest invaded cork oak, with the tool "measure line". We only used the minimum of these distances, to take into account the proximity of each tree to the closest potential invasion source.

(d) Surrounding habitat quality: Argentine ant expansion is limited by dryness and high temperatures of the scrubland matrix around cork oak trees (Angulo et al. 2011). The normalized difference vegetation index (NDVI) measures, through satellite imagery, visible and near infrared light reflected by plants, and is an estimate of the density and quality of the vegetation of a certain area (Karkauskaite et al. 2017). NDVI was measured in a circle of 100 m of radius around the trunk of the tree by accessing Landsat images of 30 × 30 pixels, using the function *create buffer* in QGIS 2.18.14 (QGIS 2013). For each tree we calculated yearly measures as the average of monthly measures (0–3 measures per month were available) and then used the average of the yearly measures from 1992 to 2000. Higher NDVI means more productivity and more vegetation cover, implying lower aridity and thus a better habitat quality for the Argentine ant.
We also measured the perimeter of each trunk (cm) at 1.5 m height, because bigger trees provide more vegetation cover, humidity and lower temperatures than smaller trees (Angulo et al. 2011).

(e) Dispersal by humans: we calculated the minimum distance from each sampled tree to the nearest path. Because humans may inadvertently transport ant queens in their cars or with their belongings, lower distances to paths would mean higher probability of ant queen transport to cork oaks. We used the same tool as in (c) to calculate the minimum distance.

(f) Dispersal by raptors: Black and Red kites (*Milvus migrans* and *M. milvus*, respectively) breed in large numbers in the study area, mainly using cork oaks and pine trees as support for their nest (Sergio et al. 2005). Both species are wide-ranging facultative scavengers, very adept at finding animal carcasses, even very small ones, such as meat items from human refuse (e.g., Kumar et al. 2018). During the breeding season, prey items are usually transported to the nest or to feeding perches, typically located within 10–50 m from the nest. We used historical data from kite surveys conducted between 1992 and 2000 (details in Sergio et al. 2005) to obtain: (1) the total number of kite nests situated in a 50 m radius centered on the trunk of each cork oak, and (2) the total number of nestlings that were raised in those nests each year. Because both variables were correlated ($R^2 = 0.88$, $p < 0.001$, $N = 37$), we chose to include in the model only the number of nests. We assumed that more nests would lead to higher probabilities of accidentally transporting queen ants that were feeding on the carrion item when this was picked up and taken to the nest area by a kite, thus dispersing the ants.

Statistical differences for each of the above independent variables were obtained with the function *drop1* and the F statistic (package stats, R Core Team 2015). We used a backward stepwise procedure in order to obtain a final model that only included statistically significant variables (Table 2).

Results

Of the total 105 cork oaks that survived the three decades of study, 67 were already invaded in 1992, 18 were invaded from 1992 to 2000 and just one more had been invaded by 2016 (Fig. 1). We also observed trees that were invaded but were found devoid of Argentine ants in subsequent years. This occurred in eight trees: six were invaded in 1992, of which three had native ants in 2000 and the other three had native ants in 2016; two trees that were invaded in 2000 had native ants in 2016.

Differences in ant community structure between invaded and uninvaded trees

The community analysis did not show significant differences in the native ant community

Table 2 Effects of anthropogenic, biotic and abiotic variables in the invasion of new trees

	Complete model		Final model		
	F	*p*	F	*p*	Sign
Species richness	2.53	0.124	17.32	< 0.001	(+)
Dominant species (%)	0.54	0.741	–	–	–
Distance to an invasion source	18.43	< 0.001	56.73	< 0.001	(−)
NDVI	0.03	0.869	–	–	–
Tree perimeter	2.10	0.159	–	–	–
Distance to a path	20.52	< 0.001	34.84	< 0.001	(−)
Number of nests	3.01	0.094	6.68	0.015	(+)
Latitude	0.62	0.440	26.12	< 0.001	(+)
Longitude	1.29	0.266	39.10	< 0.001	(+)

Statistics for the complete (initial) model and the final model, obtained by a backward stepwise procedure, are provided (N = 37). For the final model the sign of the relationship has been added

(PERMANOVA, F = 1.63, $p = 0.141$, N = 37) between the trees that were invaded or uninvaded in 2000. We ran NMDS with two dimensions and had a fair stress value of 0.05. As shown in Fig. 2a, the ant community structure of invaded and uninvaded trees was very similar, the only difference being a wider ant community in uninvaded trees, suggesting that ant communities of the invaded trees could be a subgroup of the ant communities of uninvaded trees.

The ant community of uninvaded trees significantly changed between 1992 and 2016 (PERMANOVA, F = 6.23 $p = 0.001$, N = 19, Fig. 2b). While the ant community in 1992 contains more species than in 2016, the two communities are largely overlapping, differing in some species that have been substituted for others with similar ecological functions (Table 1).

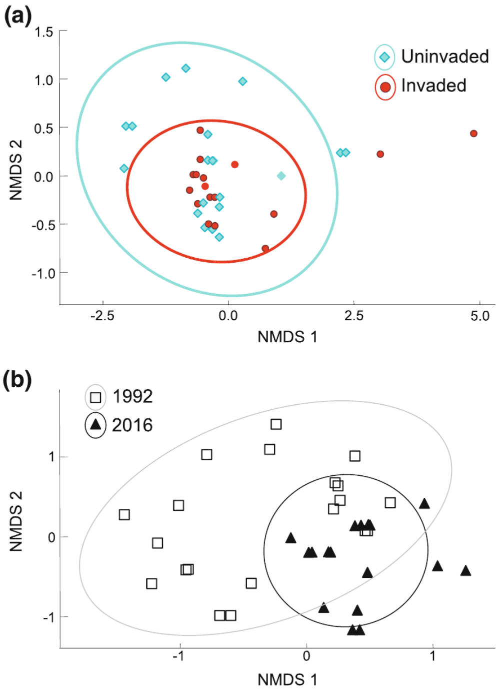

Fig. 2 Ordination of ant community similarity (NMDS) for **a** trees that were invaded by Argentine ants (red circles) or that were still devoid of the invading species by 2000 (light blue diamonds); **b** ant communities in un-invaded trees in 1992 (white squares) and in 2016 (black triangles). The ellipses represent the 95% confidence interval for each group

Anthropogenic, biotic and abiotic variables affecting the invasion of new trees

When testing the biotic resistance hypothesis, we found that species richness was selected as an important variable in the final regression model (F = 17.32, $p < 0.001$, N = 37, Fig. 3a, Table 2). However, the sign of the relationship implied no biotic resistance to invasion by the native community. Indeed, we found the opposite result: trees with higher native ant richness were more likely to be invaded. The behavioral dominance hypothesis was also not supported: the percentage of dominant species in each cork oak was not selected as a significant variable in the final model.

Geographic effects were important for the invasion process: the invasion of new trees depended on the distance to the nearest source of invasion (F = 56.73, $p < 0.001$, N = 37, Fig. 3b; Table 2). Latitude and longitude also had a significant effect, showing an invasion pattern that moved preferentially to the north

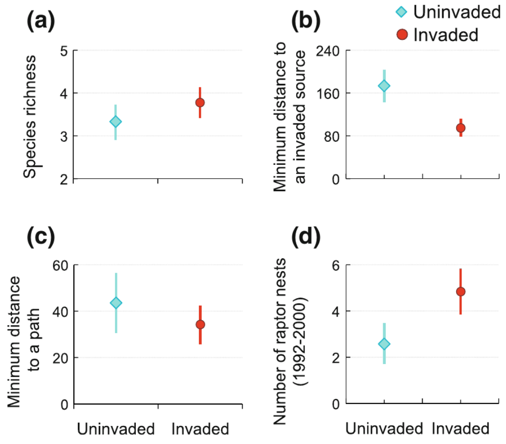

Fig. 3 Characteristics of trees invaded (red circles) or uninvaded (light blue diamonds) by the Argentine ant. **a** Average (± SE) of native ant species richness in 1992; **b** average (± SE) of the minimum distance to an invaded source (invaded tree or invasion focal point); **c** average (± SE) of the minimum distance to a path; **d** average (± SE) of total number of nests of scavenger raptors in invaded trees or in trees that remained uninvaded by 2000. Raptor nests include those of *Milvus milvus* and *M. migrans* in a 50 m circle around the tree for the period 1992–2000

and west (F = 39.10 and F = 26.12 respectively; $p < 0.001$, N = 37 in both cases, Table 2).

In relation to the tree quality and its environment, neither the perimeter of tree trunk in 1992 nor the NDVI index were selected as important variables in the final model (Fig. ESM_1, Table 2). Finally, anthropogenic and raptor facilitation were supported by our results: the likelihood of invasion increased with the proximity to a path (F = 34.84, $p < 0.001$, N = 37; Fig. 3c; Table 2) and with the number of kite nests in the immediate surroundings (F = 6.68, $p = 0.015$, N = 37, Fig. 3d; Table 2).

Discussion

We followed the invasion of *L. humile* at a local scale during three different decades (1992, 2000 and 2016), studying different biotic and abiotic factors that can influence the process, in a protected area with favorable habitats interspersed with unfavorable ones. We found that the invasion moved from main focal invasion points towards the closest trees and that the likelihood of invasion increased with the proximity to a path. The native ant community did not offer resistance to the invasion, neither in terms of species richness, nor in proportion of dominant species or community structure. However, our results suggest that raptors facilitate ant dispersal towards areas that would not be accessible to the Argentine ant by itself.

Most of the literature dealing with biotic resistance against the Argentine ant shows that native ants are not able to resist the invasion when abiotic conditions are adequate for the invader. Exceptions found in the bibliography include communities that contain dominant native species, such as *T. nigerrimum* in Corsica, which can limit the spread and establishment of this invader (Blight et al. 2010). Although *T.* cf. *nigerrimum* was found in 11% of the cork oak trees we studied, its presence did not seem to prevent invasion. *T. simrothi*, another dominant species that is ecologically similar to *T.* cf. *nigerrimum,* was shown by Way et al. (1997) to be displaced by the Argentine ant as well. Dominant species also have a role in the first steps of invasion: a higher propagule size (e.g., a larger Argentine ant colony size) is needed for successful invasion in the presence of dominant species such as *I. rufoniger* in Australia (Walters and Mackay 2005), *Pheidole megacephala* in South Africa (Mothapo and Wossler 2014) or *Monomorium antarcticum* in New Zealand (Sagata and Lester 2009).

Given the local juxtaposition of habitat conditions, the invasion of new trees in our study area could be accomplished by diffusion (e.g., "budding", where a group of workers and queens colonize new areas on foot) or by leapfrog advances, depending on whether trees are separated by favorable or unfavorable habitat (Suarez et al. 2001). Such jumps would consist of a small propagule size, and thus the effects of dominant species could have a role in preventing such new invasions. Among the five dominant species in our study area, the one that was more frequent in the trees before invasion was the native arboreal ant *C. scutellaris* (located in more than 75% of the trees). This species is aggressive and territorial, but does not always display polygyny and polydomy (two characteristics that if present could make their arboreal nests less vulnerable to Argentine ants); that may be the reason why it was systematically displaced during the invasion (Way et al. 1997; Angulo et al. 2011). When abiotic conditions are unfavorable for the invasion, native species can coexist and compete with Argentine ants for resources, thus slowing down the invasion

(Menke et al. 2007). Examples include *Solenopsis molesta, Leptothorax andrei* and *Prenolepis imparis* in Southern California (Suarez et al. 1998).

Despite the above, neither the proportion of dominant species nor species richness prevented the spread of the invader in our system. Studies in other Mediterranean climates such as the chaparral of Southern California (Menke et al. 2007) and the coastal scrub of Australia (Rowles and O'Dowd 2007) have also shown a lack of biotic resistance by native ants when abiotic conditions were favorable for the invasion. However, biotic resistance has been reported to be more effective in natural than in urban habitats (Helford 2012). Although Doñana is a natural area, the invasion is for the moment concentrated in the surroundings of a small, permanent human settlement, which could help the Argentine ant to persist against natives.

The analysis of ant community structure suggested that trees that resisted the invasion had a wider community structure than the trees that were invaded. This does not mean that they were simply composed of more species, but rather that they were organized differently, suggesting the possibility that the key to biotic resistance against invasion may not be the number of species or percentage of dominants, but rather a certain combination of species. Moreover, natural ant communities change over time, especially in isolated trees (Gove et al. 2009; Donoso 2017). In our case, ant communities of uninvaded trees differed between 1992 and 2016. The changes did not seem a result of Argentine ant invasion, but rather consisted of substitution between ecologically similar native ant species. More research should be done in order to assess this effect.

However, contrary to the biotic resistance hypothesis, our results showed that cork oaks with higher ant species richness were invaded first. Holway (1998) found that, although invasion rate was independent of native ant richness, it increased with native ant abundance. In fact, larger trees are the ones that have resources capable of maintaining more species (Kurz-Benson et al. 2006). Although in our analyses the perimeter of the tree was not a significant variable for the invasion, we also observed that larger trees had more ant species (see Fig. ESM_2a). Larger trees also support higher abundances of aphids (Ward et al. 1998), which may facilitate invasion because aphid honeydew is one of the main food sources for the Argentine ant (Shik and Silverman 2013). Both the native ant *C. scutellaris*, and the invasive Argentine ant use honeydew resources in cork oaks (Abril et al. 2007). And in our study area, ant abundance in trails on the tree trunk was correlated with the size of the tree (see Fig. ESM_2b), suggesting that bigger trees sustain bigger colonies, probably because of the greatest availability of resources, such as aphids. In addition, larger tree crowns provide more shade, which is likely to result in temperatures conducive to Argentine ant survival. Holway (2005) and Angulo et al. (2011) found that Argentine ants were more abundant in habitats with more vegetation cover and moisture, while in the scrubland they were less abundant and more likely to coexist with native species (Way et al. 1997; Holway 2005; Menke et al. 2007; Rowles and O'Dowd 2007; Roura-Pascual et al. 2011).

The presence of unfavorable dry scrubland areas separating the favorable habitat constituted by the cork oak trees has very likely slowed the expansion of the Argentine ant during these past decades. According to the review of Suarez et al. (2001), the maximum annual rate of spread of the Argentine ant invasion is 154 ± 21 m (range 15–275 m), which means that in Doñana it could have spread as far as 3.7 km (range 360–6.6 km) between 1992 and 2016. However, since 1992 only 19 of the 37 uninvaded trees in the study area were colonized, and since 2000 only one new tree was colonized, while 18 trees remain uninvaded. This occurred within a sampling area of 1.5 km radius from the main invasion focal point, where the maximum distance from the main invasion focal point to an invaded tree was 509 m in 1992. Thus, the local Argentine ant population does not seem capable of saturating its already colonized range. Moreover, we detected eight invaded trees that were subsequently devoid of Argentine ants and which were colonized again by native ants (six of these trees were already invaded in 1992 and two were invaded in 2000). This is not a rare situation. Menke et al. (2018) also found evidence of invasion retreat in Northern California, probably caused by the severe drought that the State had suffered during four consecutive years. Cooling et al. (2012) found a population collapse of the Argentine ant in New Zealand that allowed the native populations to recover, perhaps due to abiotic conditions like high rainfall or to inbreeding depression.

Although trees that were closer to the invasion source were invaded earlier, the Argentine ant has reached areas that are surrounded by unfavorable and uninvaded habitats. It is widely known that humans are dispersal vectors of the Argentine ant (Suarez et al. 2001; Ward et al. 2005; Boser et al. 2017), and proximity to paths frequently used by humans was previously suggested as a factor contributing to spread across unfavorable areas in Doñana National Park (Carpintero et al. 2005). Our results add quantitative support to this idea, showing that trees that were closer to a path were invaded earlier.

Moreover, Carpintero et al. (2005) proposed that, among vertebrates, scavenging raptors could facilitate the invasion of new trees across unfavorable habitat, and our results lend quantitative support to this hypothesis. The invasion of a tree was related to the abundance of kite nests in the surrounding habitat. Because the Argentine ant is a scavenger species (Angulo et al. 2011), it is possible to find it feeding on animal carcasses or meat refuse from humans. Queens are also sometimes present on such food items (authors' pers. obs.). When ant-infested food items are picked up and transported by kites to their nest areas, there is a chance that the propagule could be transported to an area inaccessible to the Argentine ants alone. This scenario could explain the leapfrog invasion of some isolated trees in the north of our sampling area (see Fig. 1). Although this mode of spread may be of relatively minor importance compared with the large-scale, sometimes inter-continental, human-mediated jump dispersal, it could be of importance in natural areas where large avian predators are common, where human presence is less pervasive, or where management actions have been attempted to reduce human-mediated ant dispersal. For example, at our study site, measures to prevent the inadvertent transport of Argentine ant propagules by humans have been established since 2004. These measures include substituting concrete for wood in fencing, directives for secure transport of food, garbage and other human materials from houses to the rest of the protected area, and adequate transport and conservation of organic materials used in experiments or for scientific collections. Additional routes of human-related dispersal should be taken in account when studying invasion patterns: soil movements (used for path restoration and other infrastructures), translocations of plant specimens grown in invaded areas, or constructions of underground infrastructures (which ants could use as refugia or dispersion routes to new areas). Transport by raptors cannot be prevented, especially by wide-ranging raptors such as kites that may forage in rubbish dumps tens of kilometers outside of the protected area (Heredia et al. 1991).

Independently of the local situation, the possibility that mobile vertebrate predators, such as avian facultative scavengers, can act as vectors of invasive species is relatively new in invasion ecology and adds a new dimension to invasion management and prevention. Note that some avian facultative scavengers are opportunistic-adaptable species that can be locally common in both urban and natural areas. For example, black kites are extremely abundant within urban environments of Africa and southern Asia, where they subsist almost entirely on human subsidies and refuse, reaching the highest raptor densities ever recorded (Kumar et al. 2018). Their potential as vectors of ant dispersal in these environments, or their synergy with human dispersal, would be even greater than portrayed here. In conclusion, our study lends support to previous ones suggesting that biotic resistance *per se* is unlikely to halt Argentine ant invasion, even in environments with an overall unfavourable matrix. Furthermore, colonization maintenance and spread may be supported not only by humans but also by mobile predators, whose dispersal capabilities should be considered when planning the management of invasive species.

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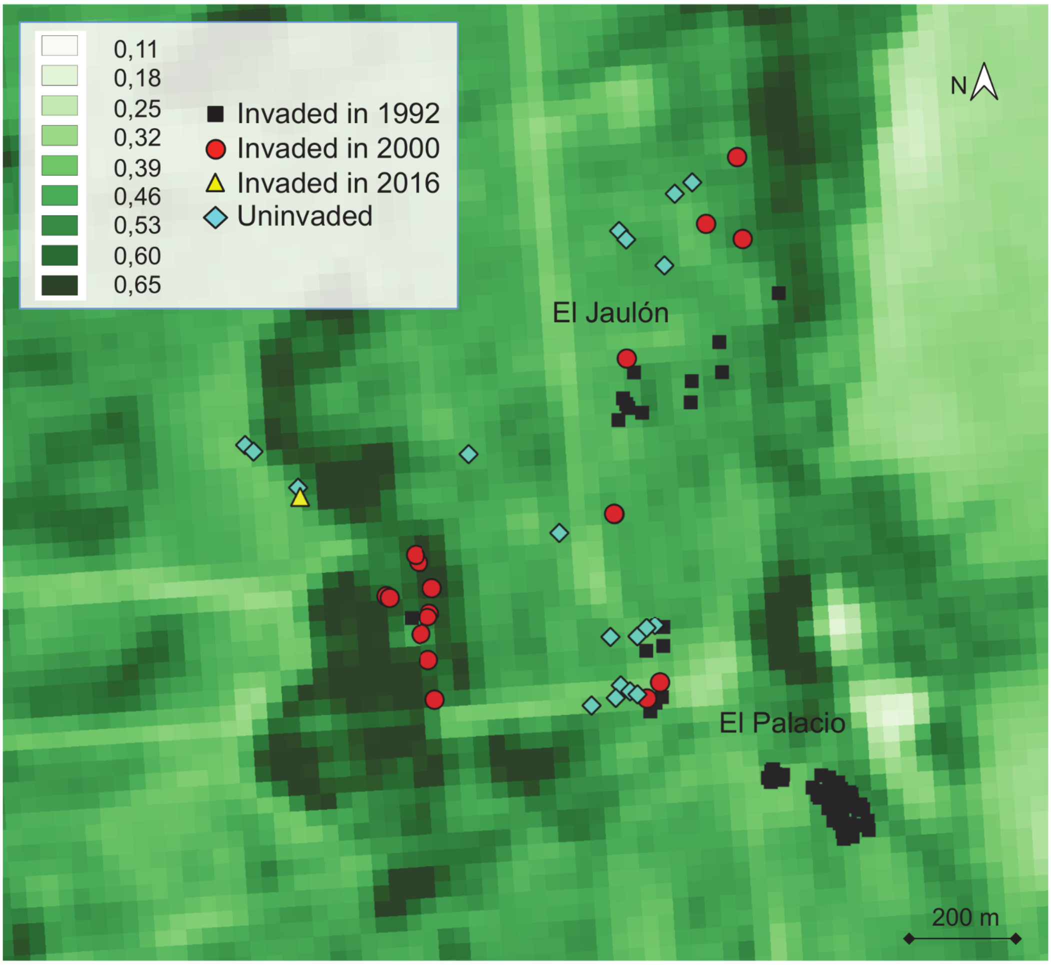

Figure ESM_1. Mean NDVI values (1992-2000) in the study area. Data is provided for 30x30 pixels. Higher values represent higher density and quality of the vegetation (see Methods). In our case higher values corresponded to trees, mainly cork oak trees, pine trees, and poplars, and to the wet scrubland, which is constituted mainly by *Erica* spp., *Ulex* spp. and *Rubus ulmifolius*. The lowest values corresponded to paths, marshlands and ponds. Intermediate values corresponded to the dry scrubland constituted mainly by *Halimium* spp., *Ulex* spp, *Cistus* spp., *Lavandula stoechas* and *Thymus mastichina*.

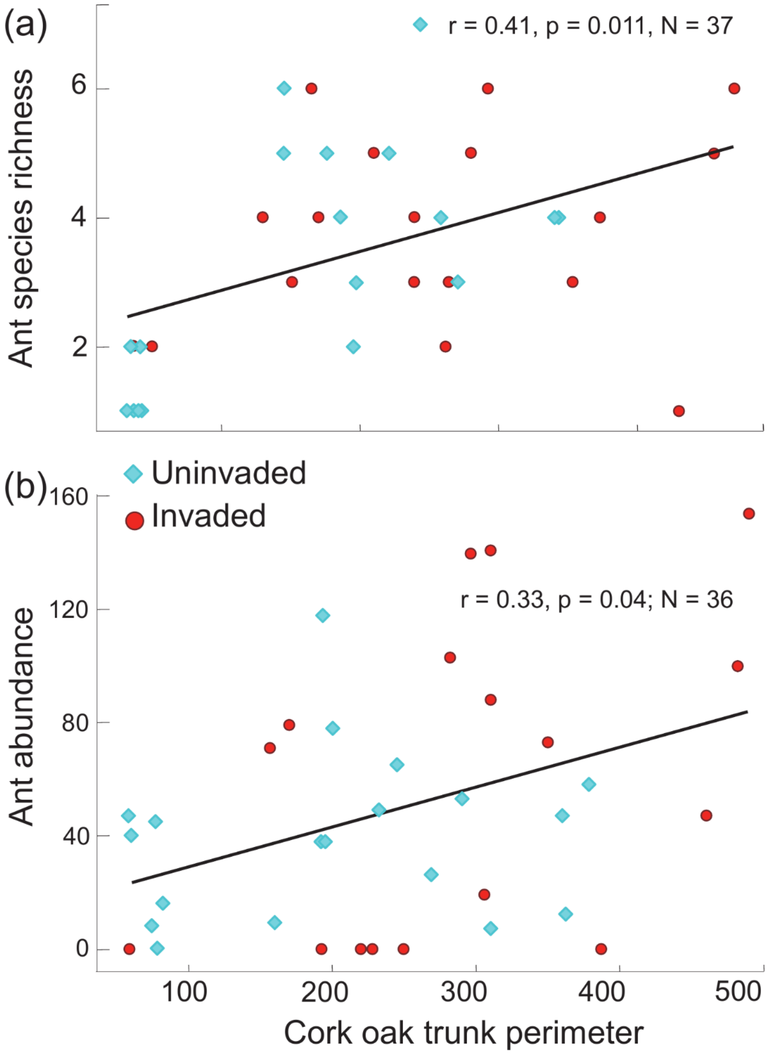

Figure ESM_2. Relationships between the size of the cork oak tree with ant species richness (a) and ant abundance (b). In (a) trunk perimeter and ant richness correspond to the year 1992 when all these trees were uninvaded (but they have been distinguished by their invasion status in 2000). In (b) trunk perimeter and ant abundance correspond to the year 2016 (we do not have ant abundance for the previous years). Ant abundance was estimated as the number of ants per minute, counted in the thickest vertical ant trail of each tree. Data comes from invaded (red circles) and uninvaded (light blue diamonds) trees; in (b) data corresponds to the abundance of Argentine ants and native ants, respectively. Native ants measured were *Crematogaster scutellaris* and *Lasius grandis* (N = 16 and N=2 respectively). We show the fit and statistics for the correlation tests performed, which were done with the cor.test function, , in the 'stats' package of R (Core Team 2015).

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