Ascertaining Key Factors Behind the Coexistence of the Native Ant Species *Plagiolepis pygmaea* with the Invasive Argentine Ant *Linepithema humile* (Hymenoptera: Formicidae)

by

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ABSTRACT

The Argentine ant, *Linepithema humile*, is a world-wide invasive ant species. Its presence has a strong negative impact on ant diversity. The present study attempts to highlight the reasons for the coexistence of this highly dominant species with *Plagiolepis pygmaea*, the only native ant species that has proved able to resist the invasion in a natural ecosystem in the north-east of the Iberian Peninsula. To quantify the aggressiveness level of both species we performed aggressiveness tests on workers in different areas: a) Argentine ant workers from areas with *P. pygmaea*, b) Argentine ant workers from areas without *P. pygmaea*, c) *P. pygmaea* from a non-invaded area and d) *P. pygmaea* from an invaded area. We also confronted Argentine ant workers with *P. pallidula* and *T. nigerrimum*. These aggressiveness tests showed that the coexistence of these two species of ants was not due to a habituation process, since the aggressiveness level observed between the four kinds of confrontations were fairly similar. We also found a lack of aggressiveness between Argentine ant workers and *P. pygmaea*, and highly submissive behavior in the latter when confronted with the invader. The peaceful character of *P. pygmaea* together with its markedly submissive behavior may be the main factors behind the coexistence of these species in the study area.

Key Words: Aggressiveness, coexistence, dominance hierarchy, *Linepithema humile*, *P. pygmaea*.

INTRODUCTION

Native to South America, the Argentine ant, *Linepithema humile* (Mayr), is a well-known invasive species (McGlynn 1999). It has spread worldwide as a result of human commercial activities in areas with Mediterranean-type

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climates (Hölldobler & Wilson 1990; Passera 1994; Suarez *et al.* 1998; Suarez *et al.* 2001) usually associated with disturbed habitats (Holway 1998a; Suarez *et al.* 2001). However, its ability to occupy natural ecosystems has also been reported (Cole *et al.* 1992; Holway 1998a; Suarez *et al.* 2001; Gómez *et al.* 2003). Where it has been introduced, the Argentine ant has impacted native ant faunas leading to changes in arthropod communities (Human & Gordon 1996; Human & Gordon 1997; Holway 1998b; Suarez *et al.* 1998), ant-vertebrate interactions (Suarez *et al.* 2000) and ant-plant relationships (Bond & Slingsby 1984; Visser *et al.* 1996; Gómez & Oliveras 2003; Blancafort & Gómez 2005). Its presence has also had negative effects on crops and plantations due to its mutualistic interactions with hemipterans, which affect the growth and production of the host plant (Buckley 1987; Ness & Bronstein 2004).

In the specific case of the Iberian Peninsula, it is found occupying areas over the entire coastal strip (Espadaler & Gómez 2003). One of these areas is a natural ecosystem in the north-eastern part of the Peninsula, specifically on the southern edge of the Gavarres massif, near the village of Castell d'Aro. In this area the invader has displaced all the native ant species present there except one, *Plagiolepis pygmaea* Latreille. (Oliveras *et al.* 2005). The mechanisms behind this coexistence remain unknown. A recent work by Grangier *et al.* (2007) reported a similar case of coexistence between native species of the *Cyphomyrmex* genus and the ant species *Wasmannia auropunctata* (Roger), a very dominant invader. In this specific case, it was found that the coexistence of these species was mediated by a process of habituation. The purpose of this study was to determine if the coexistence of *P. pygmaea* and the Argentine ant is also a result of a habituation process or is due to other factors.

MATERIAL AND METHODS

Ant colonies and sampling

In June and July 2008, we collected samples of both Argentine ant and *Plagiolepis pygmaea* colonies. Colonies of the Argentine ant were taken from two different sites. In one site it was coexisting with the native ant *P. pygmaea* and in the other there was a total absence of the native species. Similarly, colonies of *P. pygmaea* were taken from two different sites. One site was the same as that of samples of the Argentine ant coexisting with *P.*

pygmaea, and the other was a non-invaded area with a well-structured native ant community. Samples were taken from five different nests of each species in each sampling site.

We also collected workers from four different nests of the two native ant species *Pheidole pallidula* (Nylander) and *Tapinoma nigerrimum* (Nylander) in the non-invaded area.

Aggressiveness tests

In order to quantify aggressiveness between the Argentine ant and the native ant species collected, we adapted the protocol employed by Grangier *et al.* (2007). We chose at random one Argentine ant worker and one worker from the native ant species under study, and placed them in a neutral arena (diameter = 2.5 cm, height = 1 cm). The walls were coated with Fluon® to prevent the ants from escaping. The tests began with the first interaction and continued for five minutes. We noted: a) the duration of each interaction, b) the species which initiated the interaction, and c) the level of aggressiveness. The aggressiveness level was scored as follows:

Indifference: After making contact with its antagonist, the individual showed neither aggressive nor submissive behavior.

Antennation: Repeated tapping of the antennae somewhere on the other ant.

Escape: The individual moved quickly away after making contact with its opponent.

Gaster flexion: The individual raised its gaster to a vertical position as a chemical defence.

Biting: The attacker bit the body or appendixes of its antagonist.

Fight: Prolonged aggression.

We organized a total of four different categories of confrontation:

a) Argentine ant workers from nests in an invaded area where *P. pygmaea* is also present with *P. pygmaea* workers from the same invaded area.

b) Argentine ant workers from nests in an invaded area where *P. pygmaea* is also present with *P. pygmaea* workers from a non-invaded area, i.e. where there are no Argentine ants present.

c) Argentine ant workers from nests in an invaded area where there are no *P. pygmaea* present with *P. pygmaea* workers from an invaded area, i.e. where there are Argentine ants present.

d) Argentine ant workers from nests in an invaded area where there are no *P. pygmaea* present with *P. pygmaea* workers from a non-invaded area, i.e. where there are no Argentine ants present.

A total of 75 trials were performed for each combination, 15 for each nest, using different individuals each time.

To estimate the aggressiveness levels of the species in each combination, we used an aggressiveness index obtained from Errard and Hefetz (1997):

$$\frac{\Sigma^{n}_{i=1} \delta_i t_i}{T}$$

where δ_i and t_i are the interaction index and the duration of each act, respectively, and T is the total time during which the ants are in physical contact.

Data analysis

In all cases, the conditions of normality (checked using the Kolmogorov-Smirnov test) and homoscedasticity (checked using Levene's test) were not met. Data on aggressiveness were therefore statistically compared using nonparametric tests, in particular the Kruskal-Wallis test.

We used the statistics program *Statistica 6.0* (Stat. Soft, Inc., Tulsa, USA) for all analyses and graphs.

RESULTS

There were no differences in the aggressiveness index of either the Argentine ant or *P. pygmaea* in any of the confrontations analyzed (Argentine ant's aggressiveness index, Kruskal Wallis test: $H=1.89$, $p= 0.595$; *P. pygmaea's* aggressiveness index, Kruskal Wallis test: $H= 5.17$, $p= 0.1597$) (Fig. 1-2). The level of aggressiveness of the Argentine ant was higher against *P. pallidula* and *T. nigerrimum* than against *P. pygmaea* (Kruskal Wallis test: $H=119.81$; $df=2$; $P< 0.001$) (Fig.1). Similarly, the aggressiveness level of *P. pygmaea* against the Argentine ant was significantly lower than that of *P. pallidula* and *T. nigerrimum* (Kruskal Wallis test: $H=80.06$; $df=2$; $P< 0.001$) (Fig.2).

In all confrontations the frequency of non-aggressive encounters was always superior to the frequency of aggressive encounters (Table 1). While non-aggressive encounters were instigated by both the Argentine ant and *P. pygmaea* with similar frequency, aggressive encounters in all combinations of confrontations were only instigated by the Argentine ant (Table 1). The

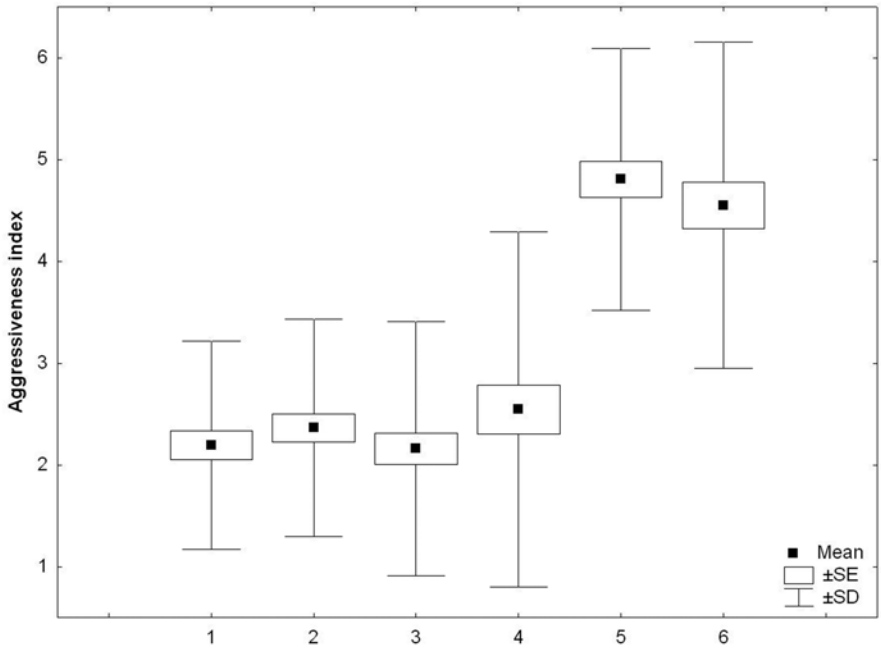

Fig. 1. Aggressiveness index of Argentine ant workers recorded during confrontations with workers from the native species studied. 1-4 corresponds to the different confrontations analysed with *P. pygmaea*, and 5-6 corresponds to the confrontations with *P. pallidula* and *T. nigerrimum* respectively.
1: Argentine ant workers coexisting with *P. pygmaea* versus *P. pygmaea* workers coexisting with Argentine ants; 2: Argentine ant workers coexisting with *P. pygmaea* versus *P. pygmaea* workers non-coexisting with Argentine ants; 3: Argentine ant workers non-coexisting with *P. pygmaea* versus *P. pygmaea* coexisting with Argentine ants; 4: Argentine ant workers non-coexisting with *P. pygmaea* versus *P. pygmaea* workers non-coexisting with the Argentine ants.

number of these aggressive encounters was significantly higher in confrontations involving Argentine ant workers from invaded areas with no presence of *P. pygmaea* than in confrontations involving Argentine ant workers from areas where these two species coexisted ($\chi^2 = 13.586$; df= 3; $P < 0.05$) (Table 1).

In all combinations of aggressive encounters, *P. pygmaea* showed itself to be markedly submissive, resorting to the use of ritualised behavior to appease its opponent. This most commonly took the form of remaining motionless while crouching after a highly aggressive confrontation in which the Argentine ant had been biting the *P. pygmaea* worker. We also observed another kind of submissive behavior adopted by the native species as a response to aggression by

Table 1. Proportion of non-aggressive and aggressive encounters carried out by *L. humile* / *P. pygmaea*.

	A vs. B	A vs. C	B vs. D	C vs. D
Non-aggressive encounters	97.97%	96.81%	93.37%	90.08%
carried out by *L. humile* / *P. pygmaea*	48.96% / 51.04%	52.63% / 47.37%	52.26% / 47.74%	47.70% / 52.30%
involving ignorance/avoidance	44.13% / 55.87%	41.45% / 58.55%	47.74% / 52.26%	44.04% / 55.96%
N	145	152	155	121
Aggressive encounters	2.03%	3.19%	6.63%	9.92%
carried out by *L. humile* / *P. pygmaea*	100% / 0%	100% / 0%	100% / 0%	100% / 0%
N	3	5	11	12

A: Argentine ant workers from areas with *P. pygmaea*. B: Argentine ant workers from areas without *P. pygmaea*. C: *P. pygmaea* from a non-invaded area. D: *P. pygmaea* from an invaded area.

the Argentine ant. It consisted of adopting a pupal posture by folding its antennae and legs in against the body and remaining motionless in this posture until the Argentine ant worker stopped its aggression. Although this submissive behavior was observed with less frequency than the former, it resulted in the same reaction on the part of the Argentine ant workers: they always stopped their aggression and ignored the *P. pygmaea* worker. The only deaths of *P. pygmaea* workers observed during aggressive encounters with the Argentine ant came about as a result of a lack of submissive behavior on the part of the native ant workers, which caused the prolongation of the aggression until their death.

DISCUSSION

Both the Argentine ant and *P. pygmaea* exhibit a similar aggressiveness index in all the confrontations analysed. This suggest that the coexistence of the two species cannot be explained as a result of a habituation process as was reported in the case of the coexistence between *Wasmannia auropunctata* and the native *Cyphomyrmex* genus (Grangier *et al.* 2007). On the other hand, Argentine ant workers demonstrate marked differences in their aggressive behavior depending on if they are confronted with *P. pygmaea* or with other native ant species such as *P. pallidula* or *T. nigerrimum*. While they show highly aggressive behavior with the latter, with the former they tend to be more peaceful, ignoring or avoiding their opponents and rarely attacking them. Similarly, *P. pygmaea* also demonstrate a lower degree of aggressiveness when confronted

with the Argentine ant than the other two species studied, which show a higher aggressiveness index when confronted with the invader. *P. pygmaea* also demonstrates very submissive behavior in confrontations with the Argentine ant, which was never observed in the case of the two native ants. These differences in aggressive behavior between the species under study in the present work can be attributed to the dominance hierarchies in ant communities. Vepsäläinen and Pisarki (1982) classified different species of ants depending on their dominance level in the ant community, establishing a rank order which consists of three categories, from species which usually win confrontations to ones that normally lose them. The first category includes very aggressive species which always defend territory consisting of their nests and a large foraging

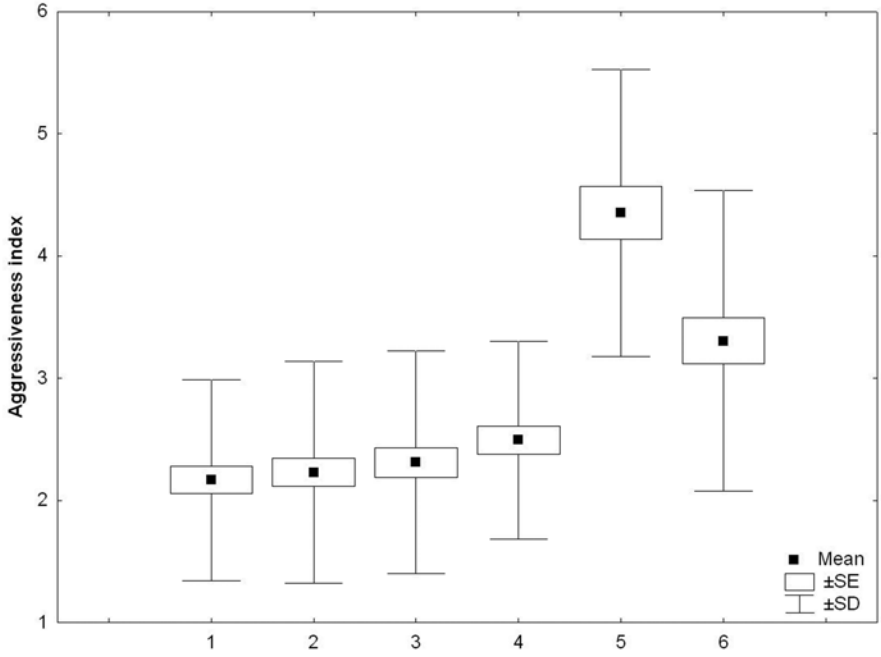

Fig. 2. Aggressiveness index of native ant species studied during confrontations with the Argentine ant. 1-4 correspond to the different confrontations analysed with *P. pygmaea*, and 5-6 correspond to the confrontations with *P. pallidula* and *T. nigerrimum* respectively.
1: Argentine ant workers coexisting with *P. pygmaea* versus *P. pygmaea* workers coexisting with Argentine ants; 2: Argentine ant workers coexisting with *P. pygmaea* versus *P. pygmaea* workers non-coexisting with Argentine ants; 3: Argentine ant workers non-coexisting with *P. pygmaea* versus *P. pygmaea* coexisting with Argentine ants; 4: Argentine ant workers non-coexisting with *P. pygmaea* versus *P. pygmaea* workers non-coexisting with the Argentine ants.

area. The second category consists of species which defend their nests and food sources, and the last comprises not very aggressive species which only defend their nests and generally avoid confrontations with other species of ants. The ranking of a species depends on both the aggression level and the dynamic density of the colony. In general, the greater these parameters, the higher the position of the colony in the hierarchy (Savolainen & Vepsäläinen 1988). In addition, species belonging to the second category tend to come into conflict with ones from the first, while local coexistence between two species belonging to the first category of dominance is unlikely due to their strong sense of territoriality (Savolainen 1990). Under this classification method, the Argentine ant, *P. pallidula* and *T. nigerrimum* belong in the first category since they behave very aggressively and have high dynamic densities (Cerdà *et al*. 1997, Carpintero & Reyes-López 2008), while *P. pygmaea* belongs to the last (Cerdà *et al*. 1997). It explains why *P. pallidula* and *T. nigerrimum* have stronger aggression indexes when confronted with the Argentine ant and vice versa, and also the low aggression level detected in confrontations between *P. pygmaea* and the Argentine ant. This lack of aggression between the Argentine ant and *P. pygmaea* contributes greatly to the coexistence of these two species. It seems that the extremely submissive behavior of the latter is the key to its resistance to the invasion. Avoiding confrontation and the use of submissive behavior to appease opponents once confrontation has been initiated are *P. pygmaea's* main weapons and what allows it to coexist with this highly dominant species. Conversely, their lack of submissive behavior and the high level of aggressiveness exhibited by the other two native ant species studied may greatly contribute to their displacement in the invaded areas.

Although it seems that the main factor contributing to the coexistence of *P. pygmaea* and the Argentine ant is the peaceful behavior demonstrated by the former, the authors think it is important to take account of the fact that Argentine ant workers from areas with no presence of *P. pygmaea* tend to initiate more aggression when confronted with this species than Argentine ant workers from areas without it. This suggests a certain degree of habituation on the part of the Argentine ant, which although not seeming to be decisive, does appear to be an additional factor underlying coexistence between these two species in invaded areas.

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