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Isabel Salado, Paloma Álvarez-Blanco, Raphaël Boulay, Olivier Blight, Sílvia Abril, et al.. The variability of iridomyrmecin, the venom of the Argentine ant, in its native and invasive ranges. *Chemoecology*, 2023, 33 (1-2), pp.17-27. 10.1007/s00049-023-00381-3 . hal-04447728

HAL Id: hal-04447728

<https://amu.hal.science/hal-04447728>

Submitted on 22 Feb 2024

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The variability of iridomyrmecin, the venom of the Argentine ant, in its native and invasive ranges

Isabel Salado¹ · Paloma Álvarez-Blanco¹ · Raphaël Boulay² · Olivier Blight³ · Sílvia Abril⁴ · Xim Cerdà¹ · Elena Angulo^{1,5}

Received: 4 September 2022 / Accepted: 20 February 2023 / Published online: 10 March 2023
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Abstract

The Argentine ant is one of the five worst invasive ants. Recently it has been shown that one of the main compounds of its pygidial gland, iridomyrmecin, is used as a venom against competitors and enemies. Here, we explore the variability in the quantities of iridomyrmecin of individual workers, along a range of locations pertaining to both its native and invasive ranges, in order to know whether its venom could have contributed to the differential invasion success of European supercolonies. We specifically compared the amount of iridomyrmecin among supercolonies in the native range and among three invasive supercolonies: the Main supercolony (the most extended worldwide), the Corsican and the Catalanian supercolonies (both with a restricted distribution in Europe). Our main result is that the variability of the iridomyrmecin is very high. Looking at mean values, we found that the amount of iridomyrmecin of the Main supercolony was the lowest while the highest corresponded to the Corsican supercolony, with the Catalanian and the native range supercolonies having intermediate values. However, variability in the values within each supercolony was similar between supercolonies. This suggests that the success of a given invasive supercolony may not be explained by higher quantities of this defensive compound. Our results open the way for exploring the connection between defensive compounds and the invasion success of this global invader.

Keywords Allomone · Biological invasions · Chemical defense · Invasive ants · *Linepithema humile* · Unicoloniality

Communicated by Thomas Schmitt.

Isabel Salado and Paloma Álvarez-Blanco contributed equally to this work.

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Introduction

In the last decades, biological invasions have become one of the main threats to biodiversity and ecosystem services in the whole world (Millennium Ecosystem Assessment 2005), leading to a global redistribution of species and driving homogenization of ecosystems (McKinney and Lockwood 1999, 2001). The Argentine ant (*Linepithema humile*, Mayr 1868) is one of the most devastating invasive ant species in the world (Holway et al. 2002). Native to South America, it has spread worldwide, mainly in regions with Mediterranean climate (Suarez et al. 2001; Wetterer et al. 2009; Vogel et al. 2010). The Argentine ant has a strong economic impact (Angulo et al. 2021) and also a heavy ecological impact on the invaded ecosystems, because it does not only displace the local arthropod fauna (Bolger et al. 2000; Holway and Suarez 2004), but it also causes a series of cascading effects in the rest of ecosystem, from plants (Christian 2001; Blancafort and Gómez 2005) to vertebrates (Suarez et al. 2000; Alvarez-Blanco et al. 2020, 2021). For these reasons, it has

been registered on the ‘100 of the World’s Worst Invasive Alien Species’ list (Lowe et al. 2000; GISD 2021).

Similar to many ant invaders, the success of the Argentine ant has been shown to rely on its social organization, known as unicoloniality, based on the formation of supercolonies. A supercolony consists of large networks of polygynous (multiple queens) genetically homogeneous nests that exhibit no aggression with each other, even if they are separated by long distances (Helanterä 2022). However, strong aggression of individuals and groups of Argentine ants is always present, both against other species (Human and Gordon 1996; Holway 1999; Buczkowski and Bennett 2008a; Abril and Gómez 2011), and also among supercolonies (Tsutsui et al. 2003; Helanterä et al. 2009; Vogel et al. 2009; Blight et al. 2012). In their native range, Argentine ants’ supercolonies are small and spatially restricted to just hundreds of meters (Pedersen et al. 2006; Suarez and Tsutsui 2008; Vogel et al. 2009; Josens et al. 2017). This is because they have to deal with interspecific competition—with the rest of the ant community—and intraspecific competition among nearby supercolonies (Vogel et al. 2009). Conversely, in its introduced range, supercolonies are much larger and allow the Argentine ant to reach high densities and spread thoroughly (Vogel et al. 2009, 2010). To date, more than 15 distinct introduced supercolonies have been found (Vogel et al. 2010; Mothapo et al. 2011).

In Europe, Giraud et al. (2002) found two distinct introduced supercolonies: the Main supercolony, which ranges over 6000 km from Italy to the Spanish Atlantic coast including Canary and Balearic Islands, and the Catalanian supercolony, which is restricted to the Spanish East coast and Balearic Islands. Blight et al. (2010) discovered a third European supercolony, the Corsican supercolony, which is localized in the South of France and the Corsica Island. All three supercolonies are expanding, but a long-term spread study of the Argentine ant supercolonies along the coast of Mediterranean islands, indicated that the Main supercolony has the highest expansion range (Castro-Cobo et al. 2021). In fact, the Main supercolony is one of the most successful invasive populations of the Argentine ant across the globe, in terms of its worldwide expansion. It has established in every continent the Argentine ant has invaded, including several countries like the USA, France, Spain, Japan, Australia, New Zealand, and South Africa (Vogel et al. 2010).

Secondary introductions could alone explain the highest spread of the Main supercolony (Bertelsmeier and Keller 2018). However, it has been shown that the Main supercolony displays a high level of aggressiveness that seems to be the cause for its ecological dominance, and, consequently, for its invasion success (Thomas et al. 2005; Sunamura et al. 2009b; Abril and Gómez 2011; Blight et al. 2017; Seko et al. 2021). The Argentine ant is known to use chemical attack in interspecific and intraspecific competition (e.g. Buczkowski

and Bennett 2008a, b; Blight et al. 2010; Abril and Gómez 2011), which appears to provide advantage in competition with other ant species (Bertelsmeier et al. 2015). Welzel et al. (2018) reported that Argentine ants use dolichodial and iridomyrmecin during aggressive interspecific interactions by applying them directly on their competitor’s surface.

Several studies have shown genetic differentiation between native and introduced populations, and among invasive supercolonies, that possibly affect recognition cues, i.e., cuticular hydrocarbons, that mediate in the nestmate distinction and determine the aggressive behaviour among them (Suarez et al. 1999; Tsutsui et al. 2000; Tsutsui and Case 2001; Giraud et al. 2002; Brandt et al. 2009). Most studies have focused on genetic, chemical and behavioural recognition approaches to characterize supercolonies, but little is known about pheromones other than cuticular hydrocarbons, or allomones such as pygidial gland secretions, of different supercolonies. These compounds play an important role in communication in social insects (d’Ettorre and Lenoir 2010). Different functions have been described, e.g. trail following and home range, sexual attraction, aggregation and dispersal, and alarm and defense (Billen and Morgan 1998).

Iridomyrmecin is a member of cyclopentanoid monoterpenes, also known as iridoids (Cavill 1969). It is produced in the pygidial gland localized in the gaster of some Dolichoderinae ants, and is mainly responsible for defensive functions (Billen 1986). Iridomyrmecin is the major component of the pygidial gland in the Argentine ant, reaching approximately 2% of its body weight (Cavill et al. 1976; Alvarez-Blanco et al. 2021). This compound was firstly isolated by Pavan (1949), who reported its bactericidal and insecticidal properties (Pavan 1952a, b). Iridomyrmecin has been linked to roles such as chemical signal in necrophoresis (Choe et al. 2009) or trail pheromone—acting together with dolichodial and (*Z*)-9-hexadecenal (Choe et al. 2012). Later, Welzel et al. (2018) revealed that pygidial gland secretions (constituted mainly by iridomyrmecin and dolichodial, in a ratio 3:1) function as defensive allomones. They cause high levels of irritation in the heterospecific competitors of the Argentine ant, such as the California harvester ant (*Pogonomyrmex californicus*). When iridomyrmecin was sprayed by the Argentine ant workers onto their opponents, the opponents lost their ability to right themselves, often resulting in turning upside down or being temporarily incapacitated. Moreover, iridomyrmecin alone has been identified to be the venom compound used by the Argentine ant to predate young amphibians (Alvarez-Blanco et al. 2021). Iridomyrmecin produced an initial paralysis of these amphibians (Alvarez-Blanco et al. 2021), which seems to be similar to the reaction of native ants shown by Welzel et al. (2018).

While it may play an important role in the ecological success of the Argentine ant, its venom has received far less attention than that of the three other invasive ants considered

poisonous (in the sense of Blum (1984)), present in the list '100 of the world's worst invasive organisms' (GISD 2021). For example, the red imported fire ant (*Solenopsis invicta*) and the little fire ant (*Wasmannia auropunctata*) inject toxins through a sting, while the yellow crazy ant (*Anoplolepis gracilipes*) sprays toxins on prey's surface (Lowe et al. 2000; GISD 2021). The venom of the Argentine ant has received little attention until recently (Welzel et al. 2018; Alvarez-Blanco et al. 2020) for several reasons. First, *L. humile* is not considered as a venomous ant species sensu stricto. The Argentine ant, like other Dolichoderinae ants, lacks a functional sting associated with poison gland (Billen 1986). Most defensive secretions come from the extremely large pygidial gland instead (Billen 1986) and are usually applied directly on enemies' surfaces (Leclercq et al. 2000; Welzel et al. 2018). Second, the high volatility of iridoids may suggest a reduced toxicity. Indeed, insecticidal effects of iridoids appear to be only temporary (Welzel et al. 2018), and some studies have claimed that iridoids show low toxicity (Cavill and Clark 1971; Attygalle and Morgan 1984), but caused paralysis and death in juvenile amphibians (Alvarez-Blanco et al. 2021). Third, the chemical composition of iridomyrmecin differs from the rest of invasive ants' venoms. Whereas *L. humile* produces iridomyrmecin, a non-glycosidic iridoid (Franzyk 2000), stinging Myrmicinae ants (e.g. *S. invicta* and *W. auropunctata*) produce alkaloids, widely researched because of their numerous applications, such as pharmaceuticals or in agriculture (Aniszewski 2015). As a result, iridomyrmecin, and iridoids in general, have received little attention in comparison with alkaloids.

Here we propose that iridomyrmecin could have contributed to the differential invasion success of the supercolonies of the Argentine ant, as it is used in aggressive confrontations against competitors. Callaway and Ridenour (2004) proposed the novel weapon hypothesis, by which some invaders carry products that could be highly efficient to compete with the community of the invaded areas, while the native community is naive to these products. In the case of the invasive Argentine ants, they could have invested in a greater quantity of iridomyrmecin to face potential competitors or predators in the invaded community. It has been recently shown that pheromone content could have favored the invasion success of the invasive *Solenopsis* spp. ants (Hu et al. 2018). In this case, we would expect that Argentine ants from invaded areas show a greater quantity of iridomyrmecin than those from native areas; and that the more successful introduced supercolonies (i.e., Main colony) would possess higher quantities of iridomyrmecin than those with limited distribution, due to its higher aggressiveness (Abril and Gómez 2011; Blight et al. 2017). Alternatively, according to the enemy release hypothesis (Elton 1958), iridomyrmecin would be less necessary in the invasive range if the native ant community is less aggressive. In this case,

we could expect higher quantity in the native range due to forced competition with native species, which includes some of the most widely distributed and destructive invasive species (*S. invicta*, *S. richteri*, *W. auropunctata*) (Suarez et al. 1999; Lowe et al. 2000; Calcaterra et al. 2008; Josens et al. 2017).

Thus, we explore differences in iridomyrmecin quantities at different levels (native vs invasive range; among invasive supercolonies; within supercolonies) in order to respond to the following questions: are iridomyrmecin quantities higher in the invasive range than in the native range? Are the most successful supercolonies those with a greater chemical arsenal (i.e. greater amount of iridomyrmecin)? Specifically, we wanted to discern whether quantities could follow the novel weapons hypothesis (more iridomyrmecin in the invaded range and in the Main supercolony) or the enemy release hypothesis (more iridomyrmecin in the native range). To discard the effect of any potential loss of the volatile compound through manipulation in the sampling process (because iridomyrmecin is used for defense and alarming when the ants are disturbed; Welzel et al. 2018), we also verified whether iridomyrmecin quantities are maintained in workers following disturbance.

Materials and methods

Disturbance experiment

In order to test whether disturbance of workers due to sampling produced a loss of iridomyrmecin, we performed an experiment to see the likely loss and recovery in the quantities of iridomyrmecin before and after disturbance. Ants were sampled in an invaded area at Doñana Biological Reserve (37°1' N, 6° 33' W; Doñana National Park, Spain) in July 2015. These ants pertained to the Main supercolony (Castro-Cobo 2021). First, we collected ten workers directly from an ant trail and considered them the control treatment for iridomyrmecin quantities. Each ant was collected independently and immediately preserved in a vial with 2 mL of hexane. To minimize disturbance, we collected them with extreme caution, approximating soft tweezers to an ant and let it climb into the tweezers without disturbing the ant trail. Then, we collected around 300 ants into an artificial colony plastic box and brought them to the lab. Then, we disturbed the ants by shaking the nest. Experiments with other ant species have used similar ways to disturb colonies (see e.g. Haight and Tschinkel 2003, with *Solenopsis invicta*). When we shook the box, we observed ants bending dorsally their gaster, a similar behavior that was described by Welzel et al. (2018) or Alvarez-Blanco et al. (2021) when Argentine ants were presented against an opponent. After shaking the box, we sampled ten workers at each of these times: 0 min, 1 h,

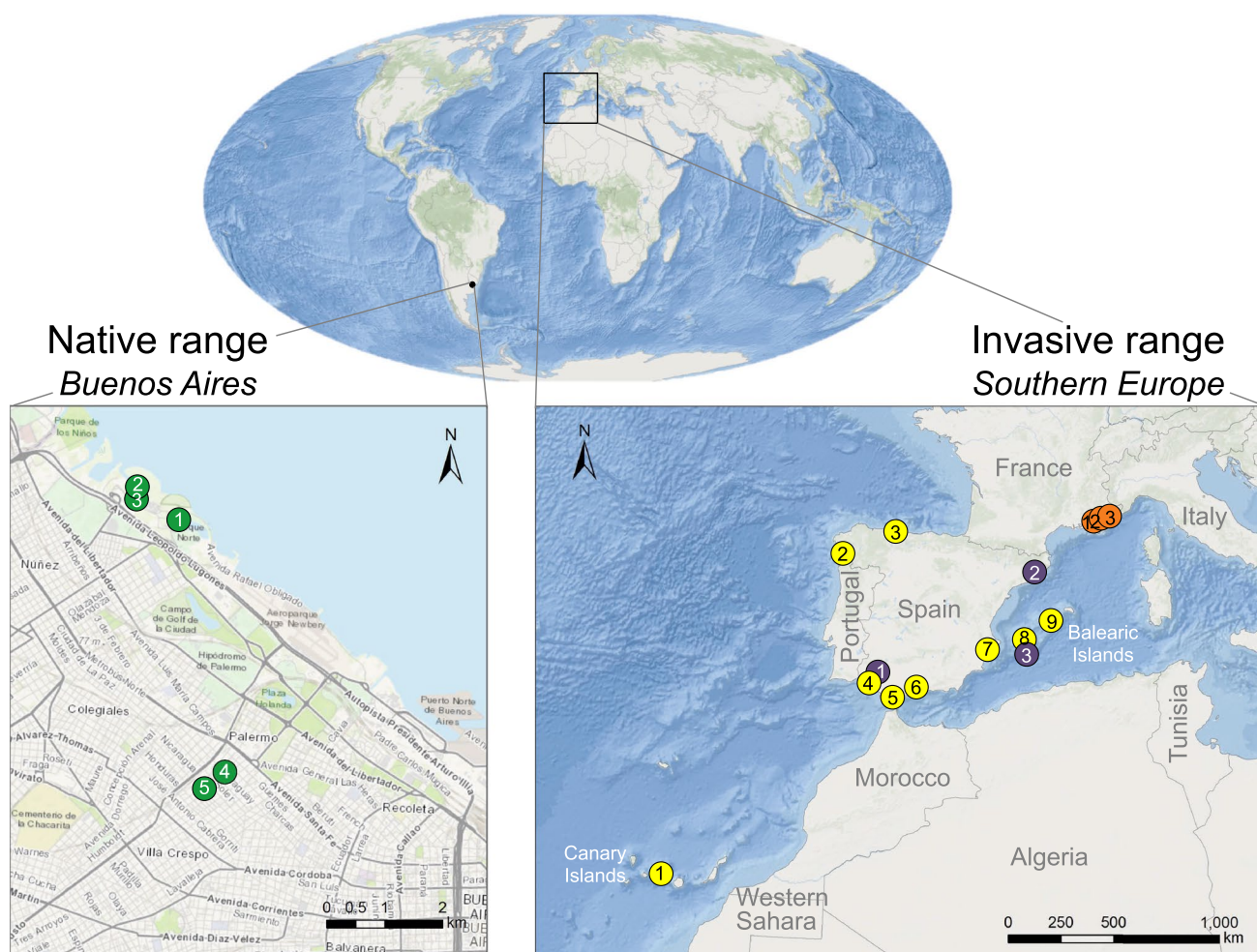


Fig. 1 Map of study area and sampling points. Supercolonies of the native range in Buenos Aires are numbered in green, and in the invasive range, different regions of the Main supercolony are in yellow,

regions of the Catalanian supercolony are in purple, and nests of the Corsican supercolony are in orange

12 h, 24 h, 2 days, 4 days, 7 days and 14 days. The colony fragment was maintained in laboratory conditions and the ants were fed with mealworm larvae, small crickets and diluted honey. We wore gloves when manipulating the samples and cleaned the tweezers with cotton soaked in alcohol after the collection of each worker.

Sampling among and within supercolonies

Argentine ants were obtained from their original range at Buenos Aires (Argentina) and from invaded areas in the Iberian Peninsula, Canary and Balearic Islands (Spain) and southern France, which included the Main, the Catalanian and the Corsican supercolonies (Fig. 1; Online Resource 1). In the native range, at Buenos Aires, ants from five colonies were sampled, which are known to be highly aggressive among them. For the Main and Catalanian supercolonies, we sampled nine and three regions respectively. Regions were at

least 50 km apart or in different islands, along the Mediterranean and Atlantic coast of the Iberian Peninsula and in the Balearic and Canary Islands. Within each region, we collected more than one nest when possible, which were at least 500 m apart from each other. For the Corsican supercolony, we sampled the three known nests in southern France.

The sampling was done with the same procedure described above for the control treatment in the disturbance experiment: one ant was collected each time without disturbing the ants of the track and immediately preserving them in 2 mL-vial of hexane. We collected a minimum of 10–15 ants per nest.

Assignment to the Main, the Catalanian or the Corsican supercolony was based on the results of one-to-one interactions, following a protocol designed by X. Espadaler (Castro-Cobo et al. 2021). In some localities there was knowledge on which supercolony the nests belonged to, based on previous work on these areas (Blight et al. 2017;

Castro-Cobo et al. 2021). We performed encounters in Petri dishes confronting two individual workers, one of a known supercolony (Main, Catalanian or Corsican) and the other of the unknown supercolony; five repetitions are enough to show whether the unknown supercolony pertains or not to the known supercolony if the encounters are peaceful or aggressive, respectively.

Chemical analyses

Chemical compounds were extracted in the hexane in which each ant was sampled in the field. Iridomyrmecin levels were analysed using gas chromatography (GC/FID—Shimadzu 2010 equipped with a 30 m × 0.25 mm i.d.-BPX5, 0.25 mm capillary column). Helium was used as the carrier gas (flow rate of 35.1 ml/min). The injection port and detector temperatures were set to 280 °C and 310 °C, respectively. The GC oven was programmed to heat at a rate of 10°C/min from 60 to 300 °C, with a 1 min initial hold and a 20 min final hold. Decyl-alcohol (99%) was used as the internal standard, and the calibration curve for quantifying iridomyrmecin concentrations in the samples was constructed using synthetic iridomyrmecin (Chauhan and Schmidt 2014). The calibration curve was obtained with 4 to 7 different concentrations of iridomyrmecin, according to the iridomyrmecin content of the samples. Concentrations were obtained by a two-fold serial dilution, starting at a 5.93 mg/mL of compound (iridomyrmecin and hexane). Compound quantifications were then determined by calculating the area under the peak relative to the internal standard for the different samples and corrected by the calibration curve. Manual peak integration and the calibration curve were performed using GC-solution software v.2.3. Shimadzu 2010.

Ant size

After chemical analysis, an estimation of the size of each ant was obtained. The inter-ocular distance was measured under a stereomicroscope (Zeiss Stereo Discovery v.8). Inter-ocular distance is a measurement of head width (it is the maximum head width measured at the interocular line). Head width is a standard and accurate measure of overall body size in ants (Hölldobler and Wilson 1990; Kaspari 1993) and inter-ocular distance is considered as a prioritized trait to be measured in ant communities (Parr et al. 2017).

The influence of body size on the quantities of iridomyrmecin is, to our knowledge, unknown, as well as the variation in body size of the Argentine ant between supercolonies. Body size could influence the amount or production of venom, such as in different species of the genus *Solenopsis* in which venom amounts increase with body size (Brand et al. 1973; Haight and Tschinkel 2003; Haight 2008; Fox et al. 2012). We performed a preliminary analysis to detect

whether there is variability in the Argentine ant body size among supercolonies. We performed a linear mixed-effects model using the “*lmer*” function of the *lme4* package (Bates et al. 2015) in R software (R Core Team 2022), with the nest as a random factor. To evaluate the supercolony effect, we used the “*anova*” function from the *lmerTest* package (Kuznetsova et al. 2017) and we showed that there are significant differences in ant size among supercolonies ($F = 3.82$, $p = 0.017$, $N = 660$). Iridomyrmecin quantities were subsequently size-adjusted on the following analysis, by using the quantities found in each individual with respect to its size.

Statistical analyses

In the disturbance experiment, we wanted to know if iridomyrmecin quantities changed after disturbance of the ants: quantities could decrease if they were used as a defense to the disturbance and then recuperate after the disturbance. Size-adjusted iridomyrmecin quantities (dependent variable) were analysed using linear models employing a Gaussian distribution, using the “*lm*” function of the *stats* package in R software (R Core Team 2022). Time after disturbance was the fixed independent variable. To evaluate the treatment effect, we computed an ANOVA of the same R package. Both, residual normality and variance homogeneity, were checked with Shapiro–Wilk test and Breusch–Pagan test, respectively.

We wanted to know whether there were differences in iridomyrmecin quantities of individual workers, among the populations and supercolonies sampled in Europe and in the native range. The following models were performed for testing differences in the size-adjusted amount of iridomyrmecin among: (i) Native supercolonies collected in the native range; (ii) Main supercolony regions; (iii) Catalanian supercolony regions; (iv) Corsican supercolony nests and (v) Supercolonies, including the native (Buenos Aires supercolonies) and the invasive range (Main, Catalanian and Corsican supercolonies). In the cases (i) and (iv), we performed a linear model (with square root transformation of the response variable in (i) to fulfill normality assumptions). In the cases (ii) and (iii) we used general linear mixed-effects models with gamma distribution and log-link function, and in (v) we used linear mixed-effects model (the response variable was square root transformed); in these mixed models the covariance of ants pertaining to the same nests was taken into account by including the nest as a random factor. Linear models were fitted using the “*lm*” function in the R package *stats* (R Core Team 2022), linear mixed models were fitted with “*lmer*” function and general mixed models using “*glmer*” function, both in the R package *lme4* (Bates et al. 2015). In order to test the effect of each independent variable, we used an anova for linear models (F statistic), “*drop1*” function for general linear mixed models, both from

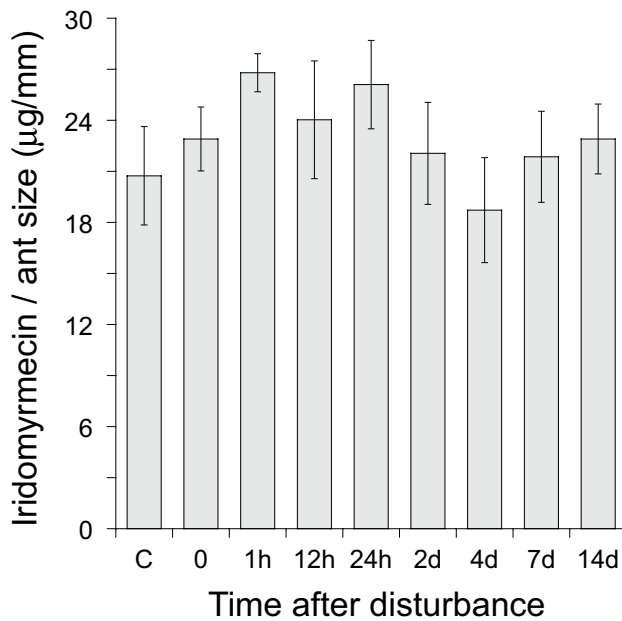


Fig. 2 Changes in size-adjusted iridomyrmecin quantities (mean \pm SE) following disturbance. Time after disturbance corresponds to: 0, just disturbed; 1, 12, and 24 h after disturbance; 2, 4, 7 and 14 days after disturbance. The control (C) corresponds to workers sampled from trails without disturbance, prior to collection of the colony fragment for the experiment

the *stats* package (R Core Team 2022), and “*anova*” function from the *lmerTest* package (Kuznetsova et al. 2017). The “*drop1*” function compares the model including and excluding the variables based on the likelihood-ratio test statistics (*LRT*, option test = “*Chi*”). Finally, when significant differences were found, we performed pairwise post-hoc comparison, using the *lsmeans* package (Lenth 2016). In the analysis comparing the invaded and native supercolonies, beside pairwise comparisons among Main, Catalanian, Corsican and native supercolonies, an a priori contrast was defined in order to compare the three invasive supercolonies to the native supercolonies using the “*glht*” function in the *multcomp* package (Hothorn et al. 2008). Analyses were performed under R version 4.2.2.

Repeatability was tested for GC-Shimadzu, manual peak integration and ant size measurements following Senar (1999) (Online Resource 2).

Results

Disturbance experiment

Size-adjusted iridomyrmecin quantities were not affected by disturbance ($F = 0.94$, $p = 0.487$, $N = 88$, Fig. 2). Therefore,

disturbance of workers did not produce significant changes in iridomyrmecin quantities.

Differences in iridomyrmecin among supercolonies in the native range

Size-adjusted iridomyrmecin quantities did differ among the five supercolonies in the native range of *L. humile* collected in Buenos Aires ($F = 3.07$, $p = 0.022$, $N = 73$, Fig. 3, Online Resource 1). Post-hoc Tukey test did not show differences among supercolonies, but a contrast test without any correction gave differences among them (Fig. 3; Online Resource 3a).

Differences in iridomyrmecin within supercolonies

In the Main supercolony, size-adjusted iridomyrmecin quantities were different depending on the region ($LRT = 28.95$, $p < 0.001$, $N = 421$, Fig. 3, Online Resource 1). Post-hoc comparisons showed significant differences among some of the regions within the wide range occupied by the Main supercolony although differences did not seem to be related with geographic proximity (Fig. 3; Online Resource 3b). In the Catalanian supercolony, iridomyrmecin quantities differed among regions ($LRT = 10.11$, $p = 0.006$, $N = 108$). Region located on the Pitiusas islands, was the one showing significant difference with the other two continental regions (Fig. 3; Online Resource 3c). In the Corsican supercolony, iridomyrmecin quantities did not differ among nests ($F = 2.53$, $p = 0.089$, $N = 58$).

Differences in iridomyrmecin among supercolonies

Finally, when we compared the supercolonies (with four categories: Main, Catalanian, Corsican and native range), we obtained that size-adjusted iridomyrmecin quantities varied among them ($F = 6.04$, $p = 0.002$, $N = 660$). When comparing native and invaded ranges, the a priori contrast did not show differences in size-adjusted iridomyrmecin quantities ($p = 0.495$, Online Resource 3d). Pairwise comparisons showed significant differences only in the invasive range between the Corsican and the Main supercolonies ($p = 0.003$, $N = 479$) with the Corsican having the highest iridomyrmecin value and the Main supercolony having the lowest value. There were no differences between the Catalanian and the other two invasive supercolonies (Fig. 3; Online Resource 3d).

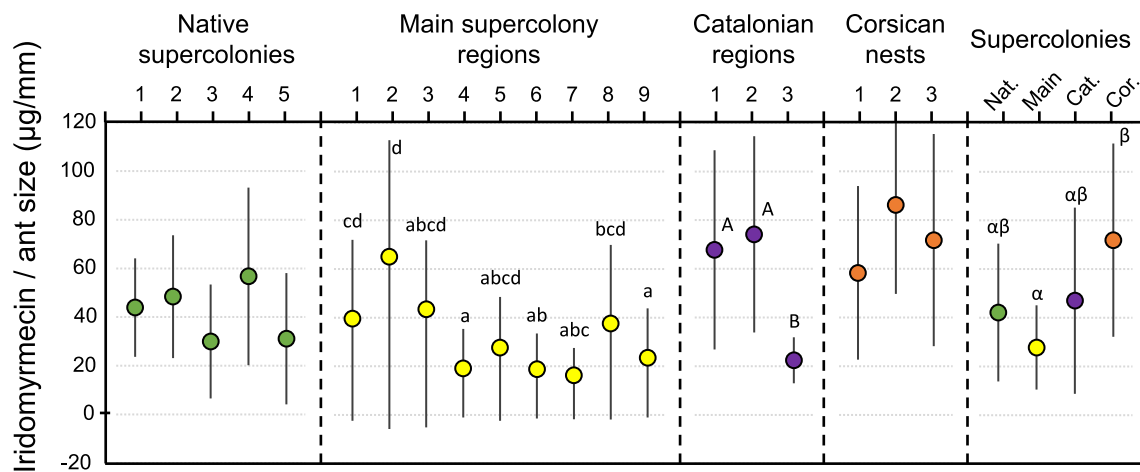


Fig. 3 Size-adjusted iridomyrmecin quantities (mean \pm SD) among supercolonies of the native range (green), among different regions of the Main supercolony in the invasive range (yellow), among different regions of the Catalonian supercolony in the invasive range (purple), among different nests of the Corsican supercolony (orange)

and among supercolonies (green, yellow, purple and orange for the Natives, Main, Catalonian and Corsican, respectively). Numbers in each supercolony correspond to sampled locations shown in Fig. 1. Post-hoc groups are shown when significant differences were found in the statistical models

Discussion

We assessed whether the quantities of iridomyrmecin, one of the main defensive compounds of the invasive Argentine ant, could vary between native and invaded areas, and contribute to the differential invasion success among different supercolonies in Europe, with the assumption that the venom contributes to the success of the invasion. Contrary to what we expected, we observed no difference in iridomyrmecin amounts between the native and the invasive ranges. Moreover, the amount of iridomyrmecin was highly variable within supercolonies, but differences did not seem to be related to the invasion success of the three invasive supercolonies studied here.

Initially, differences between Argentine ant supercolonies were described using genetics. The first studies focused on the genetic differences between native and invasive supercolonies (Suarez et al. 1999; Tsutsui et al. 2000; Tsutsui and Case 2001), and then among supercolonies in the invasive range in Europe (Giraud et al. 2002), in Japan (Hirata et al. 2008), or worldwide (Vogel et al. 2010; Inoue et al. 2013). Later, cuticular hydrocarbons were used to identify different supercolonies in the Argentine ant, e.g., two in South Africa (Mothapo and Wossler 2011), three in Europe (Blight et al. 2012), four in Japan (Sunamura et al. 2009a) and supercolonies worldwide (Brandt et al. 2009; Vogel et al. 2009). Supercolonies have also been reported in other invasive ant species, such as *Lasius neglectus* (Ugelvig et al. 2008), *Anoplolepis gracilipes* (Thomas et al. 2010; Drescher et al. 2010), *Pheidole megacephala* (Fournier et al. 2009), *Wasmannia auropunctata* (Errard et al. 2005), *Monomorium pharaonis* (Schmidt et al. 2010) and *Nylanderia fulva* (Eyer

et al. 2018), and in non-invasive ants, such as *Formica paralugubris*, or *Cataglyphis niger* (Holzer et al. 2006; Saar et al. 2014).

In some invasive and native ant species, venoms have also been explored between castes, populations, species or environments. For example, *Solenopsis* species differ not only in their cuticular hydrocarbons but also in the patterns of their alkaloid venoms, and within a nest, different castes have a different ratio of two venom components (Brand et al. 1973; Fox et al. 2012; Hu et al. 2018). In the native ant *Odontomachus haematodus*, venom composition has been observed to vary with geographic distance (Touchard et al. 2015); in the native ant *Ectatomma brunneum*, venom composition was significantly different between individuals from different environments (Bernardi et al. 2017); and in the genus *Pseudomyrmex*, the ratio of two venom components differed in species with different nesting sites (Touchard et al. 2014). However, there is a lack of studies comparing venoms of different supercolonies in invasive ant species.

Blight et al. (2012) highlighted the importance of conducting more qualitative and quantitative analyses around the aggression behaviour between supercolonies. Our results showed that there are no relevant differences among supercolonies with regards to the main defensive compound of the Argentine ant. The high variability among supercolonies irrespective of their geographic origin is in agreement with other works. For example, high levels of phenotypic plasticity have been suggested to account for behavioral differences between supercolonies independently of their origin (native or invasive supercolonies (Blight et al. 2017)). Felden et al. (2018) experimentally increased behavioral plasticity and found that the increased foraging activity or aggression was

similar in the native and invasive range of the Argentine ant. Following the same reasoning as Felden et al. (2018), it seems that iridomyrmecin variation is also conserved along the introduction of the Argentine ant in different locations and along the different supercolonies examined here.

Ways forward and limitations

Two main points could be approached in further studies to understand the possible contribution of the pygidial components to invasiveness: the quantification of the venom at the colony level and the quantification of other compounds in the pygidial gland. In our study, we measured total quantities of iridomyrmecin of individual Argentine ants. Welzel et al. (2018) showed that the Argentine ant uses all the quantity of chemical compounds stored in its pygidial gland in agonistic encounters. However, defense and alarm are colony-level functions that usually act together, carrying out an effective and coordinated group defense. *Linepithema humile* could be taking advantage of this alarm function of iridoids by investing in a numerical increase at a population level instead of increasing iridomyrmecin quantities at individual level (Cavill and Clark 1971; Attygalle and Morgan 1984; Welzel et al. 2018).

An increase of pygidial glands products could occur more frequently in invasion fronts, where invasive Argentine ants are in direct contact with native species. It has also been shown that other life history traits change in these border areas (with respect to already invaded areas), such as the size of the nest or the number and the size of the queens (Abril et al. 2013; Diaz et al. 2014). Moreover, iridomyrmecin quantities may have also been maintained only in the invasion fronts. Thus, iridomyrmecin quantities at invasion fronts should be explored to determine factors affecting iridomyrmecin variation at the local and population context, instead of individual variation.

In our analysis, we measured only one compound, the iridomyrmecin. Although iridomyrmecin is the major component of defensive secretion of *L. humile* (Cavill and Houghton 1974; Welzel et al. 2018), and it is responsible for the paralysis of competitors and predators (Alvarez-Blanco et al. 2020), pygidial glands contain a battery of defensive compounds that act together. For example, dolichodial is another volatile iridoid in the pygidial gland secretions that is also used during aggressive interactions (Cavill et al. 1976; Welzel et al. 2018). Other compounds can help iridomyrmecin to reach and stay in the target, such as iridodials (Attygalle and Morgan 1984), and it is also probable that other non-volatile and/or more polar compounds take part in defensive secretions (Welzel et al. 2018).

Finally, there is no knowledge about the patterns of natural synthesis of these chemical products, or how their amount varies with different factors, such as worker age

or size, colony structure, or temporal and/or spatial variation. For example, in *S. invicta* the synthesis is restricted to young workers and there is higher production in spring than in other seasons (Haight and Tschinkel 2003). In the same species, body size correlated with venom dose but not with the number of stings delivered by a worker (Haight 2008). Here we detected significant variation in worker size among supercolonies, however, the mechanisms explaining these differences should be explored.

Growing concerns about the effects of alien species in recipient environments have claimed to give top priority to those alien species with potentially high environmental impacts in order to distribute management resources efficiently (Blackburn et al. 2014; Kumschick et al. 2015; Booy et al. 2020). Considering the strong environmental impacts caused by the Argentine ant in invaded ecosystems and its worldwide distribution, the Argentine ant is likely one of the most urgent cases on which to focus research and management efforts. Our study opens the way of exploring the contribution of defensive compounds in the competitive ability and spread of this global invader.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00049-023-00381-3>.

Acknowledgements Ana Carvajal, Charlotte Hemingway, Sara Castro-Cobo, Miguel Lozano, Dailos Hernández-Brito, Sebastián Palacios, Roxana Josens, Nuria Morrow, María del Carmen Ramírez, Francisco Miranda and Isabel Afán. ICTS-RBD-CSIC provided logistic support during fieldwork. We thank laboratory facilities at Estación Biológica de Doñana (Laboratorio de Ecofisiología, LEF; Laboratorio de Ecología Acuática, LEA; Laboratorio de Procesado de Muestras, LPM; and Laboratorio de Sistemas de Información Geográfica y Tel-detección, LAST). We thank two anonymous reviewers for their helpful comments.

Author contributions EA and PA-B came up with and designed the study. EA, XC, SA, and OB carried out the field sampling and PA-B carried out the disturbance experiment. IS, RB and PA-B performed the chemical analyses and the statistical analyses; EA, IS and PA-B analyzed the data and wrote the paper and all authors reviewed the text.

Funding Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature. Funds came from the Spanish MINECO and FEDER (CGL2013-43660-P, CGL2015-65807-P). Spanish MINECO also funded E.A. (RyC postdoctoral fellowship), P.A-B. (predoctoral fellowship [BES-2013-064713]) and I.S. (JAE-Intro fellowship [JAEINT17-EX-0526]).

Data availability Data used for these analyses can be found in the supplementary material (Supplementary Data).

Code availability Code used for these analyses is available in the supplementary material.

Declarations

Conflict of interest The authors declare no competing interests.

Consent for publication All authors agreed to the submission of the final manuscript.

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