Diversity and Distributions

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Newcomers and Old Friends: Long-Distance and Bridgehead Introductions Both Contribute to the Recent Invasion of the Little Fire Ant in Southern Europe

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ABSTRACT

Aim: Biological invasions result from the combination of (i) population dispersal opportunities and (ii) adaptations to the recipient environment. Identifying complex migration histories, made of long-distance dispersal from the native range and secondary introductions, or genetic patterns indicative of adaptation is crucial to build coherent management efforts of problematic invasive species. We here aimed at determining the routes of introduction and describing the genetic peculiarities of recent introductions of *Wasmannia auropunctata*, a destructive invasive ant species with a polymorphic clonal/sexual reproduction system.

Location: We focused on three recently established European populations (Cyprus, France and Spain) and their relationship with earlier worldwide introductions and the native South American range of *W. auropunctata*.

Methods: We used a combination of cytochrome *c* oxidase subunit I (COI) sequencing and genotyping of a total of 686 European individuals at 12 microsatellite loci (from 76 nests and seven localities), together with previous worldwide datasets totalling 503 COI sequences and 6963 genotyped individuals. Phylogenetic reconstruction and genetic differentiation analyses were used to infer the origin, reproduction systems and genetic diversity of the three European populations.

Results: We show that the history of the invasion of Europe is a mix of secondary introductions from a Mediterranean bridgehead population (Israel), and a novel long-distance introduction from a climatically similar area of northeastern Argentina. All newly introduced populations reproduce clonally and display an outbred genotypic pattern, consistent with all prior introductions and with anthropised areas of the native range.

Main Conclusions: This study confirms that preventing novel introductions is complex and requires adequate surveillance tools to simultaneously monitor areas of the native range with potential prior adaptation and previous introductions that could act as bridgeheads. Because of their ecological and genetic similarities, the fate of more ancient introductions could help us foresee what the future holds for European introductions.

1 | Introduction

Biological invasions represent a major threat to ecosystems worldwide, altering crucial ecosystem processes and accelerating the loss of biodiversity (Roy et al. 2023; Simberloff et al. 2013), in synergy with other components of global change. International initiatives such as the Convention on Biological Diversity have long recognised the large impacts of biological invasions on the environment and human livelihood (Diagne et al. 2021; Pyšek et al. 2020). The introductions of invasive alien species (IAS) that lead to their spread and dominance in a novel geographical region (i.e., successful introductions) are often facilitated by the adequation between their biological traits, shaped by their evolutionary history in their native region, and the environmental conditions of the recipient region (Facon et al. 2006). Human activities, because they simplify and homogenise ecosystems worldwide (Baiser et al. 2012; McKinney and Lockwood 1999; Newbold et al. 2015), increase the likelihood that species simultaneously adapt to anthropised ecosystems of their native and introduced ranges. Additionally, recent waves of globalisation of international trade have multiplied opportunities for human transport of non-native species (Hulme 2009; Seebens 2019). As a result of these effects of human activities, biological invasions have surged in the past century, and the urgency of mitigating their occurrence cannot be overstated (Hulme 2021).

One of the keys to preventing biological invasions and their detrimental effects is to carefully monitor the routes of introduction as well as the ecological and evolutionary processes that are instrumental to historical and present establishments of IAS (Dlugosch et al. 2015; Estoup and Guillemaud 2010). Introductions of IAS are increasingly recognised as complex events, both temporally and spatially, where a succession of simple steps can lead to intricate histories (e.g., see Eyer et al. 2021). First, introductions have long been seen as (un)fortunate long-distance dispersal events, often associated with genetic bottlenecks (Sakai et al. 2001; Sax et al. 2005; Simberloff 2009; Tsutsui et al. 2000). While these primary long-distance introductions play a pivotal role in large-scale invasions, they now seem much more frequent and less fortuitous. Recurrent events along the same or multiple routes can increase propagule pressure on some environments, countering the putative decrease in the genetic adaptive potential of small founding populations. Second, previously established introduced populations can act as 'bridgehead' populations, leading to secondary introductions (Floerl et al. 2009; Lombaert et al. 2010). Along transportation routes, major hubs—acting as large, heavily connected nodes—are particularly vulnerable to early invasions and are likely to initiate further introductions. Finally, both primary introductions from the native range and secondary introductions from the introduced range can interact, and genetic admixture has been regularly shown to participate in maintained or increased genetic diversity in the introduced range relative to the native range (Dlugosch and Parker 2008; Uller and Leimu 2011). It is worth noting that adaptive potential can result from a preliminary build-up in specific ecosystems of the native range prior to dispersal, and in particular in human-altered areas, a scenario that was termed 'Anthropogenically Induced Adaptation to Invade' (AIAI; Hufbauer et al. 2012). Deciphering such potentially intricate histories of movement and adaptation lies at the heart of designing management strategies informed on demonstrated invasion risks, to curb the environmental and socio-economical costs of biological invasions.

Among IAS that incur tremendous costs on societies worldwide, insect pests rank among the worst, propagating diseases, consuming crops and harming infrastructures (Bradshaw et al. 2016). In particular, invasive ant species feature several traits, such as supercoloniality, affinity for disturbed environments and expansion through independent colony founding (Holway et al. 2002; Passera 1994; Wong et al. 2023), that account for their invasiveness and detrimental impact on agricultural, environmental and welfare sectors (Angulo et al. 2022; Gruber et al. 2022; Lach and Hooper-Bùi 2009). Over 300 ant species have established outdoor populations away from their native range (Wong et al. 2023). While the exact ecological impacts of most of these species are understudied, 36 non-native ant species are considered of global concern (Gruber et al. 2022; Lowe et al. 2000; Rabitsch and Blight 2021). Among these, a recent survey demonstrated that two species accounted for most of the overall damage: the red imported fire ant, *Solenopsis invicta* Buren, and the little fire ant, *Wasmannia auropunctata* (Roger) (Angulo et al. 2022). Both of these species have been the focus of studies of their invasion histories worldwide (Ascunce et al. 2011; Foucaud, Orivel, et al. 2010), but recurrent new records illustrate their continuous progression in many parts of the world (Montgomery et al. 2022; Wetterer and Porter 2003). The red imported fire ant, native to South America, has spread across the world in less than one century, but it was only recently detected in Europe (Sicily, Italy; Menchetti et al. 2023), with evidence suggesting its presence in the region dating back to at least 2015 (Menchetti et al. 2024). *Wasmannia auropunctata*, originating from northern South America (Chifflet et al. 2018; Cuezzo et al. 2015), has been notified in new areas of Africa (Jallow and Gomez 2024) and of the Asia–Pacific region, such as in Guam (Raymundo and Miller 2012), mainland China (Chen et al. 2022; Wang et al. 2024) and Taiwan (Hsu et al. 2022; Lee et al. 2021). Its presence in the Mediterranean area dates back further, with an introduced Israeli population detected in 2005 (Vonshak et al. 2010), but new reports have piled up in recent years: Spain in 2018 (Espadaler et al. 2018, 2020; Pradera and Espadaler 2024; Arcos et al. 2025; Pérez-Delgado et al. 2025) and both Cyprus (Demetriou et al. 2022) and

France (Blight et al. 2024) in 2022. The arrival of *W. auropunctata* in several countries of the European Union constitutes a grave threat to natural, urban and agronomical environments, as was acknowledged by its addition to the list of species of Union concern in July 2022 (Blight 2020; Rabitsch 2022; EU 1203/2022). While the origin of the Israeli population is well described (Rey et al. 2012), with a single introduction from central eastern Argentina—a bioclimatic region mainly with temperatures similar to its introduced area—there has been no comprehensive study yet on the origins and relationships between all Mediterranean introductions of *W. auropunctata* so far (but see Coulin et al. 2019).

Interestingly, there is slightly more to *W. auropunctata* invasions than prior adaptation (e.g., to the lower temperatures in a higher latitude) followed by long-distance dispersal, and that is because of its peculiar reproductive biology. Most ant species reproduce via a haplodiploid reproduction system, where diploid females (both reproductive queens and infertile workers) are produced through sexual reproduction and haploid males are produced through arrhenotokous parthenogenesis. This system is similar to classical sexual reproduction in that it promotes recombination and the genetic mixing of male and female gene pools. In *W. auropunctata*, this reproduction system co-exists with a rather uncommon clonal reproduction system, where female queens reproduce via thelytokous parthenogenesis, males via androgenesis (a female-controlled clonal production of males) and only female workers are sexually produced (Foucaud, Estoup, et al. 2010; Foucaud et al. 2006, 2007; Fournier et al. 2005; Rey et al. 2011, 2013). Unlike sex, this clonal reproduction system blocks most of the recombination and separates males and queens' gene pools, while retaining a possible outbreeding effect in workers. This clonal reproduction system has been linked to increased heterosis and thermal tolerance in workers (Coulin et al. 2019; Foucaud et al. 2013; Rey et al. 2012) and occurs at a higher rate in anthropogenic areas of the native range (Chifflet et al. 2018; Foucaud et al. 2007, 2009). So far, invasive populations worldwide only consist of clonal populations (Chifflet et al. 2018; Foucaud, Orivel, et al. 2010; Hsu et al. 2022).

With regard to the threat posed by these recent introductions to the ecosystems and socio-economic welfare of European and Mediterranean countries, this study aims to address two main questions. First, are newly introduced populations the result of multiple independent long-distance introductions, new bridgehead introductions or a combination of both? In more detail, what are the geographical origin(s) of the *W. auropunctata* populations that have been established in Spain, Cyprus and France, and what are their genetic relationships? Second, from an adaptive perspective, do these recently introduced populations exhibit a specific genetic makeup indicative of an AIAI scenario? In particular, do introduced European populations of *W. auropunctata* display a specific reproductive system and/or genetic outbreeding relative to previously known native and invasive populations? The answers to these questions will help us inform the current assessment of these introductions in Southern Europe in two key ways. On one hand, they will pinpoint specific trade routes that may require regulatory actions. On the other hand, they will offer insights into the future of these introductions, and in particular their evolutionary potential in the longer term.

2 | Methods

The origins, system of reproduction and genetic makeup of recently introduced European populations were investigated using genetic resources in the form of (i) mitochondrial sequences and (ii) microsatellite markers, as described below.

2.1 | Field Collection

Recent introductions of *W. auropunctata* in Cyprus, France and Spain were sampled at seven locations in spring 2023 and one in autumn 2024. The French introduction, previously uncovered in a single neighbourhood in Toulon (Blight et al. 2024), now consists of two sites in the Provence region, approximately 60 km apart (Figure 1). The Spanish introduction consists of three distinct sites of a few acres located in the Marbella, Estepona and Mijas Costa municipalities of the Málaga province, Andalucía (Figure 1; Espadaler et al. 2018, 2020; Pradera and Espadaler 2024; but see also Arcos et al. 2025; Pérez-Delgado et al. 2025). For both countries, research of new establishments of *W. auropunctata* in the vicinity of introduction areas using visual inspection and baiting proved unsuccessful, confirming the relatively restricted spatial extent of those introductions so far. Conversely, the spatial extent of the Cyprus population of *W. auropunctata* appears much larger and has still to be determined in its entirety (Demetriou et al. 2023). In our study, sampled Cyprus locations included the originally detected sites of Mesogi (Paphos district) and Limassol (Demetriou et al. 2022), as well as a site in Neo Chorio, on the Northwestern coast of Cyprus (Akamas Peninsula; Figure 1).

In each of these locations, at least two and up to 17 nests (i.e., an aggregation of workers, brood and/or queens) were sampled using visual inspection and baiting for a total of 76 nests sampled (Table 1). All specimens were immediately preserved in ethanol at their time of collection. Because of their value in providing direct proof of genetic relationship to retrace introduction routes, a particular emphasis was placed on retrieving both males and female sexuals (queens). In total, we sampled 505 female sexuals, 65 male sexuals and 155 sexual larvae, together with hundreds of female workers, specifically for this study.

2.2 | Microsatellite Genotyping

The microsatellite genotyping was carried out following Foucaud et al. (2006). Briefly, DNA was extracted using a standard Chelex protocol from at least seven female sexuals (mean = 35 ± 5), all males and at least 38 workers per site, leading to a final dataset of 276 female sexuals, 65 males and 345 workers overall Europe (Table 1), genotyped at 12 microsatellite loci (Fournier et al. 2005). For certain comparisons, we additionally used previously published genotyping datasets for the same 12 loci, encompassing 2916 individuals from the native range of *W. auropunctata* (Foucaud et al. 2009; Foucaud, Orivel, et al. 2010), and 3361 individuals from worldwide introductions (Foucaud, Orivel, et al. 2010; Vonshak et al. 2010), including 330 individuals from Israel. Results from Monte Carlo simulations following the methods of Foucaud et al. (2006) indicated that our 12 microsatellite kit was sufficient to prove

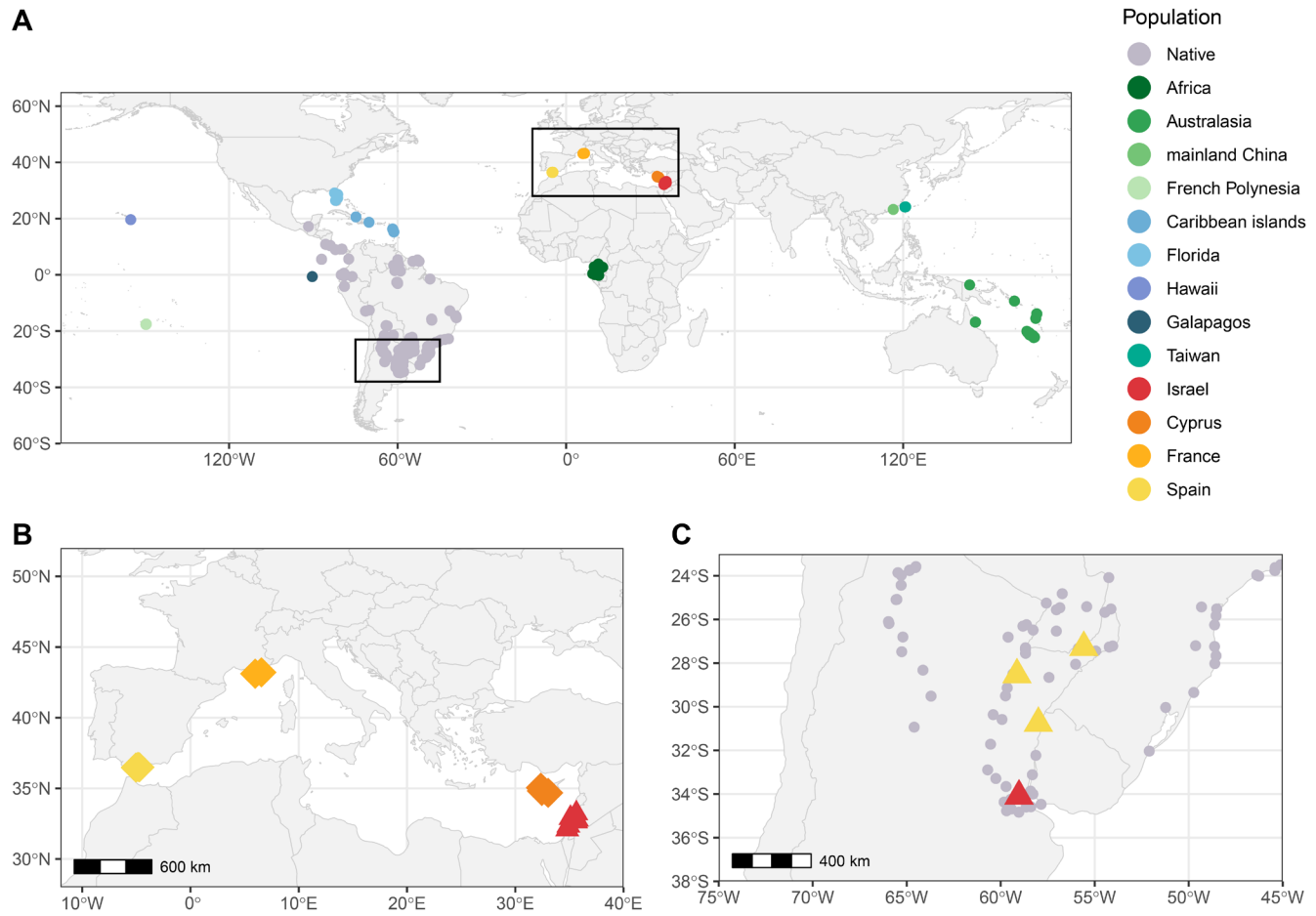

FIGURE 1 | Map of the sampling location. (A) Locations of native (grey) and introduced (coloured) *Wasmannia auropunctata* populations whose genetic material (mtDNA and/or microsatellites) was included in the present study. (B) All known populations established in the Mediterranean area were sampled (Israel: Red triangles, Cyprus: Dark orange diamonds, France: Light orange diamonds, Spain: Yellow diamonds). (C) In the Southern part of the native range, the locations of one and three populations whose mitochondrial DNA matched with those of the Israel group (Israel, Cyprus, France) and of the Spanish introduction, respectively, are indicated with triangles of matching colours.

the genetic identity of all inferred groups of clonal genotypes at the $p < 10^{-6}$ level.

2.3 | Mitochondrial Dataset

The mitochondrial dataset comprised 503 samples in total and was constructed from 284 COI sequences already available in GenBank (244 of which have been produced by the co-authors of the study), 91 in BOLD, 110 sequences that have been used in previous publications but not published on GenBank (Foucaud et al. 2006; Rey et al. 2012) and 18 sequences newly produced for this study, as follows. Following the same DNA extraction protocol as above, we used HCO (Folmer et al. 1994) and LCO universal primers with an alternative internal reverse (Rey et al. 2012) to amplify a 658 bp fragment of the COI gene. PCR products were purified and sequenced by Eurofins Genomics (Ebersberg, Germany). All previously undeposited and newly produced sequences were deposited on GenBank (accession numbers PQ466606–PQ466733). Table S1 compiles GenBank/BOLD accession numbers and locations of the 503 COI sequences used in this study. All sequences were aligned using MAFFT v.7 (Katoh et al. 2019) with default settings. The COI dataset was further visually inspected with Mesquite v.3.70 in order to detect the presence of potential stop codons; it consists of 503 sequences of a maximum length of 658 aligned characters, of which 169 are variable (including 137 parsimony-informative sites).

2.4 | Phylogenetic Analyses and Haplotype Network Reconstructions

Phylogenetic analyses were carried out under maximum likelihood (ML), as implemented in IQ-TREE v.2.2.6 (Minh et al. 2020). The mitochondrial COI dataset was divided into three partitions (one per codon position), with best-fit substitution models for each partition selected using the Bayesian information criterion through ModelFinder (Kalyaanamoorthy et al. 2017). The best-scoring ML tree was obtained using a heuristic search implementing 500 random-addition replicates, with the following settings: random-starting tree, thorough hill-climbing nearest neighbour interchange (NNI) search (*-allnni -nstop 500* option), a perturbation strength of 0.5 (*-pers 0.5* option), partition-resampling strategy (*–sampling GENE* option) and best partition scheme allowing the merging of partitions (*-m MFP+MERGE* option). Clade support was assessed using

TABLE 1 | Genotypic sampling of European populations of *W. auropunctata* (in number of individuals genotyped at 12 microsatellite loci).

Range	Date of 1st observation	Country	Site	Sampled nests	Number of genotyped				References
					Queens	Males	Workers	Total	
Introduced	2018	Spain	Marbella	17	48	4	42	94	This study
Introduced	2018	Spain	Mijas Costa	16	53	15	40	108	This study
Introduced	2018	Spain	Estepona	16	47	17	44	108	This study
Introduced	2022	Cyprus	Mesogi	4	26	3	44	73	This study
Introduced	2022	Cyprus	Limassol	4	25	3	50	78	This study
Introduced	2022	Cyprus	Neo Chorio	4	28	8	40	76	This study
Introduced	2023	France	Toulon	13	39	15	47	101	This study
Introduced	2023	France	Croix Valmer	2	7	0	38	45	This study
			Total (sampled)	76	273 (502)	65 (65)	345 (784)	683 (1361)	
Introduced	2005	Israel		29	56	43	230	329	Vonshak et al. (2009)
Native		Argentina		85	75	17	138	230	Rey et al. (2012)
Native		Brazil		66	250	135	527	912	Foucaud et al. (2009), Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Native		French Guiana		111	286	225	1169	1680	Foucaud et al. (2009), Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Native		Costa Rica		8	25	6	63	94	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	1959	Cameroon		55	196	79	250	525	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	1914	Gabon		19	57	44	145	246	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	1924	Florida		11	26	8	88	122	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	*NA*	Cuba		2	5	0	23	28	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	*NA*	Guadeloupe		10	2	0	75	77	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)

(Continues)

TABLE 1 | (Continued)

Range	Date of 1st observation	Country	Site	Sampled nests	Number of genotyped				References
					Queens	Males	Workers	Total	
Introduced	*NA*	Dominican Republic		1	0	0	8	8	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	*NA*	Dominica		1	0	0	8	8	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	1902	Cocos Island		1	2	0	7	9	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	1935	Galapagos		1	0	0	8	8	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	1972	New Caledonia		82	580	206	702	1488	Foucaud et al. (2006)
Introduced	1995	Tahiti		9	69	45	71	185	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	1999	Hawaii		9	16	0	71	87	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	1998	Vanuatu		10	18	2	71	91	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	2002	Australia		7	14	10	54	78	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	2005	Papua New Guinea		3	4	0	23	27	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
Introduced	1974	Solomons		1	13	0	32	45	Foucaud, Orivel, et al. (2010), Foucaud, Estoup, et al. (2010)
			Total	521	1694	820	3763	6277	

1000 replicates for both SH-like approximate likelihood ratio tests (SH-aLRT; Guindon et al. 2010) and ultrafast bootstraps (uBV; Minh et al. 2013). Nodes corresponding to SH-aLRT values ≥80% and uBV ≥95% were considered strongly supported following authors' recommendations. The root for the resulting tree (best-fit ML tree with clade support values) was determined using two outgroup-free rooting methods: (i) midpoint rooting (MPR) as implemented with FigTree v1.4.4 (available at http://tree.bio.ed.ac.uk/software/figtree/) and (ii) minimal ancestor deviation (MAD) using the MAD program (Tria et al. 2017).

To provide a complementary overview of *Wasmannia* relationships, a haplotype network was also reconstructed with the same mitochondrial dataset using the median joining algorithm implemented in POPART v1.7 (Leigh and Bryant 2015), with default settings (all sequences were kept, and an epsilon value of 0 was used).

2.5 | Reproduction System and Genotypic Patterns of Introduced European Populations

We characterised the reproductive systems and the relationships between genotypes by investigating individual microsatellite genotypes visually and using a custom R script. This script was first used to identify clones (i.e., identical multilocus genotypes) in a given sample of genotypes, using the *poppr* package (Kamvar et al. 2014, 2015). We considered two genotypes to be clonal when they shared identical multilocus genotypes at 12 microsatellite loci, or when they differed either (i) by only one dinucleotide repeat at one of the 12 genotyped loci (as this pattern is likely to correspond to one mutational event at a microsatellite locus) or (ii) by homozygosity for one allele at a single heterozygous locus of the clonal queen genotype (likely corresponding to a recombination or gene conversion event during thelytoky; Foucaud et al. 2006). In addition to characterising reproduction systems, detection of clonality can also confirm routes of introduction, in the case where populations share identical clonal queen and/or male genotypes.

In addition to investigating microsatellite genotypes from the Mediterranean area ('Mediterranean microsatellite dataset'; Table 1), we inferred broader scale genetic relationships between worldwide populations of *W. auropunctata* ('worldwide microsatellite dataset'; Table 1). First, we evaluated the genetic proximity of worldwide populations with a Principal Component Analysis (PCA) framework using the *adegenet* package (Jombart 2008). Second, we computed pairwise genetic distances between individual genotypes using a variant of Chakraborty and Jin's allele-shared distance (Chakraborty and Jin 1993), as defined in Fournier et al. (2005). Distance matrices were then used to construct dendrograms from individual genotypes using the Neighbour-Joining algorithm (Saitou and Nei 1987) from the *ape* package (Paradis and Schliep 2019). Complementary to this approach, we performed Bayesian clustering analyses using STRUCTURE (Pritchard et al. 2000) as detailed in the associated Appendix S1.

The worldwide microsatellite dataset was also used to investigate specific genotypic patterns. Previous studies indicated that *W. auropunctata* populations differed according to their outbreeding (i.e., their large difference between male and female gene pools): more similar males and females were the signature of sexual populations of the natural areas within the native range, while mainly clonal populations of anthropogenic areas of both native and introduced ranges harboured strikingly dissimilar male and female genotypes (Foucaud et al. 2009). Overall, this pattern results in significantly more heterozygous workers in introduced and native clonal populations than in native sexual populations, and was linked to invasive status and thermal tolerance (Foucaud et al. 2013; Rey et al. 2012). To investigate this pattern in introduced European populations and compare them to previously known native and introduced populations worldwide, we computed two basic population genetic statistics indicative of outbreeding (i.e., observed heterozygosity and mean difference in allele size within and between multilocus genotypes). Within-individual heterozygosity, Ho_w, was computed as the number of loci of an individual genotype showing different alleles. Within-individual difference in allelic size, DS_w, was computed as the difference in base pairs between the two alleles at a given locus of a single individual genotype, averaged over loci. Ho_w and DS_w statistics were computed for every locus, and we calculated their means for every population. We then tested for statistical differences in mean Ho_w and DS_w values: (i) between native populations, introduced populations except the Mediterranean zone and Mediterranean populations using a non-parametric Friedman test and (ii) between pairs of these populations using Conover posthoc tests adjusted using the Holm method.

3 | Results

3.1 | Origin of Introduced European Populations

Both MPR and MAD approaches infer the same root for the best-fit ML mitochondrial COI tree, which recovers two main highly supported (uBV and SH-aLRT of 100%) clades (hereafter named clade A and B; see Figure S1), consistent with the results of previous studies (Chifflet et al. 2016; Mikheyev and Mueller 2007; Rey et al. 2012). Clade A contains mainly haplotypes found in Caribbean Islands, Florida, Galapagos, Hawaii, Taiwan and parts of Australasia (Papua New Guinea, Vanuatu, Australia), while Clade B contains the European haplotypes and haplotypes found in mainland China, Africa, French Polynesia and Australasia (New Caledonia). European haplotypes, though originating from clade B and showing close similarity to those from earlier introductions in Australasia, French Polynesia, Africa and mainland China, remain distinct from these other clade B haplotypes (corresponding to the H1 haplotype from subclade B-IV in Chifflet et al. 2016), and even more from clade A introductions, such as Taiwan, Galapagos or some Caribbean islands. Cyprus and France introductions are characterised by a single shared haplotype, which is identical to the unique Israel haplotype and to an Argentinian haplotype from the Las Tejas site in Zárate, Buenos Aires province (corresponding to the H7 haplotype of subclade B-IV in Chifflet et al. 2016; Figure 1B,C). The Spanish introduction is also characterised by a single shared haplotype, identical to three haplotypes all located in northeastern Argentina: one from Puerto Ocampo (Santa Fe province), one from Chajarí (Entre Ríos Province) and the last one from San Ignacio (Misiones Province), corresponding to the H4 haplotype

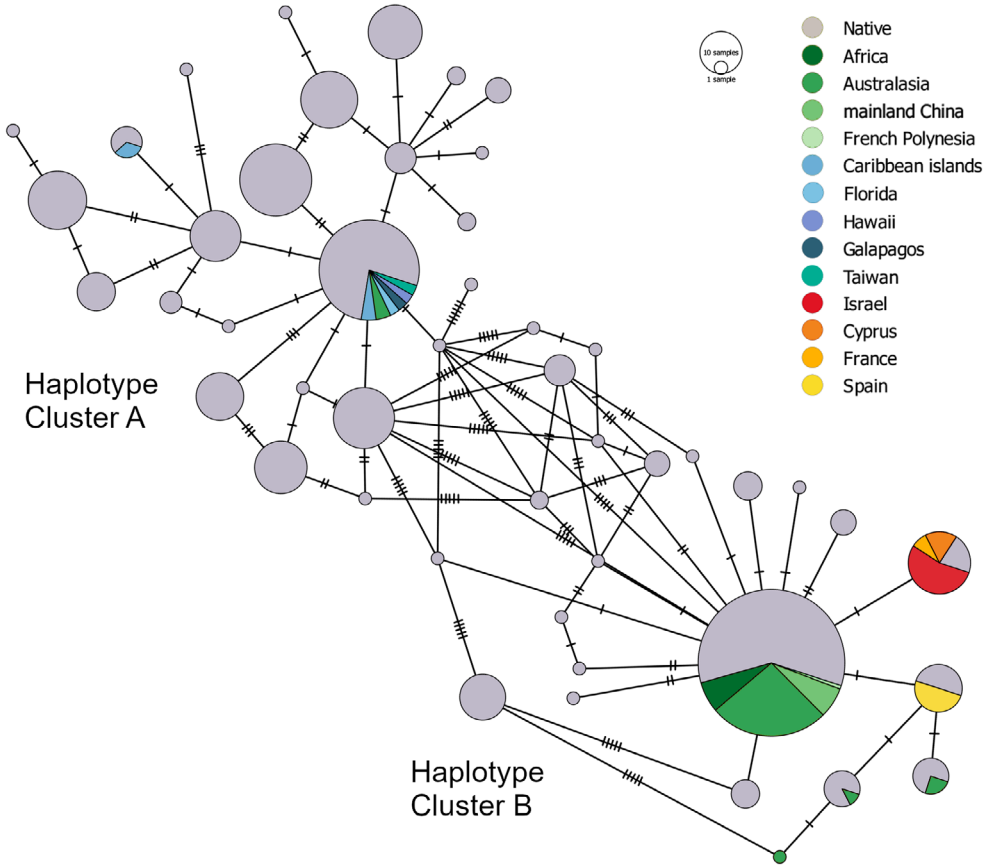

FIGURE 2 | Haplotype network of native and introduced *W. auropunctata* populations. Each circle represents a haplotype, circle size and colour represent haplotype frequency and origin, respectively (the two main haplotype clusters are also highlighted and labelled A and B). Hatch marks indicate the number of mutations. Native (grey) areas of the circles also containing Israel, Cyprus, France (red, dark and light orange), and Spain (yellow) haplotypes are all from Argentina (Zarate locality, and Puerto Ocampo, Chajarí and San Ignacio localities, respectively).

from subclade B-VI in Chifflet et al. (2016; Figure 1B,C). These three sites are located between 300 and 500 km apart from each other and at least 400 km north from the known origin of the Israeli introduction.

The results of the analyses of the two microsatellite datasets help us to better understand some of the details of these introductions. First, analyses of the Mediterranean dataset show that the Cypriot and French introductions harbour genotypes that are identical to each other and to the Israeli introduction (see below for details). This signal is particularly strong because of the extremely low number of alleles by locus (two to three, see below) in these populations. While several nests in Las Tejas have been genotyped, and appear to share more alleles with Israeli, Cypriot and French introductions than other native populations, none so far have been found to be identical to these introductions. Second, analyses of the worldwide microsatellite dataset confirm that the Spanish introduction is completely distinct from other Mediterranean introductions (Figures 1B,C and 2). Both Principal Component Analysis and distance-based dendrograms confirmed that Israel, Cyprus and France form a single genetic group, while the Spanish introduction appears genetically closer to previously known native populations from Argentina and neighbouring countries (Figure 3). These results were confirmed by our Bayesian clustering analyses (see Appendix S1).

3.2 | Reproduction System and Genotypic Patterns of Introduced European Populations

Regarding their reproduction system, all introduced European populations displayed the clonal reproduction system typical of *W. auropunctata*. In Cyprus, 73 of the 82 genotyped queens shared an identical genotype with the Israeli clonal queen genotype. In France, 37 of the 39 genotyped queens shared it with the Israeli and Cypriot clonal queens. In Spain, 142 of the 148 genotyped queens shared an identical genotype (but different from the Israeli, Cypriot and French clonal queens). Eight of the 14 Cypriot males and 13 of the 15 French males were found to be identical to the Israeli clonal male genotype. Finally, 30 of 36 Spanish males shared an identical genotype, which was different from the clonal male genotype found in Israel, Cyprus and France. All workers were found to be produced sexually, as is typically the case in all populations (i.e., clonal or sexual) of *W. auropunctata*. As a result, workers from Israel, Cyprus and France are genetically indistinguishable, in contrast to the genetically distinct Spanish workers (Figure 3).

Interestingly, the genotyping of such a large number of individuals (276 queens and 65 males) also enabled us to detect several genetic features that were already noticed in previous studies of the introduced range of *W. auropunctata*. First, among the clonal queens presented above, we detected rare instances of

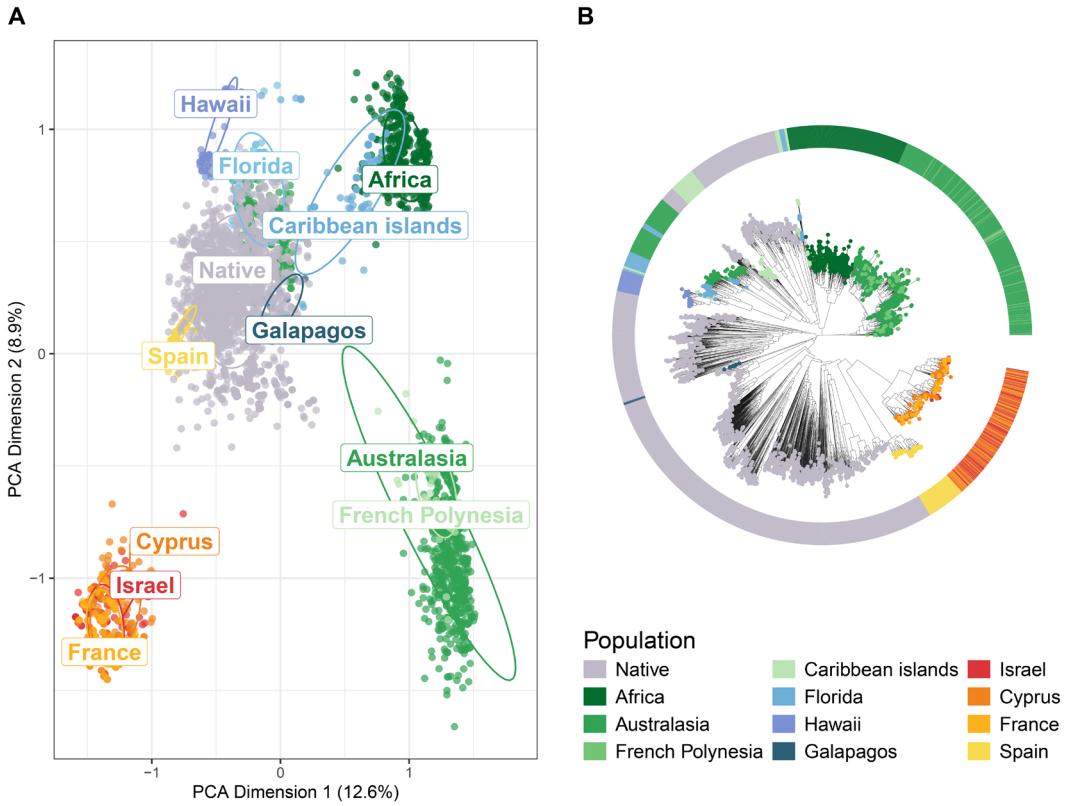

FIGURE 3 | Genetic relationships between worldwide populations of *W. auropunctata*. (A) PCA results indicate that Israel, Cyprus and France cluster together and away from other worldwide populations, while Spain regroups closer to previously sampled native populations. (B) Dendrogram using allele-shared distance illustrates the same genetic signal, with Israel, Cyprus and France mixed together and away from Spain. Colours represent populations as in Figure 1.

recombination events (one in a Cypriot clonal queen) and mutational events (one and five in Cypriot and Spanish clonal queens, respectively, and three and two in Cypriot and French clonal males, respectively). Second, we detected queen and male genotypes demonstrating the occurrence of rare sexual production of new sexuals: nine queens were produced sexually from the main male and female genotypes (six in Cyprus, two in France and one in Spain) and three Spanish clonal queens were derived from sexually produced queens (termed Q2 queens in previous studies of the reproduction system of *W. auropunctata*; Foucaud et al. 2006). In males, three Spanish males were found to be sexually produced by the main Spanish clonal queen genotype.

The genotypic patterns of all introduced populations in the Mediterranean area partly followed the overall pattern observed worldwide. Sexually produced workers of introduced queen and male clones showed high levels of heterozygosity in all introduced Mediterranean populations (range 0.81–0.87; Figure 4). Workers' heterozygosities were sometimes found to be significantly elevated when compared to native or even other introduced populations (Friedman test: $F_5 = 19.188$; $p = 0.002$; posthoc Conover tests: Israel vs. Native/Introduced: all $p < 0.035$; France vs. Native/Introduced: all $p < 0.004$). This was not the case for the Cyprus and Spanish populations, due to the presence of sexually derived clonal lineages that mix male and female allelic pools and negatively impact workers' heterozygosity. Interestingly, differences in allele size in workers were not more elevated in European introductions than in native populations (Friedman test: $F_5 = 3.241$; $p = 0.66$), contrary to the global pattern uncovered worldwide.

4 | Discussion

4.1 | Historical Complexity of European Introductions of *W. auropunctata*

Our genetic analyses offer unambiguous results about the origin of all novel introductions of *W. auropunctata* in Southern Europe. First, Cypriot and French introductions both display identical mtDNA and microsatellite genotypes to the Israeli introduction, which originated from Argentina before 2005, at least 10 years earlier (Rey et al. 2012; Figures 1B,C and 2). This result is highly suggestive of both Cypriot and French introductions being secondary ones, with the Israeli introduction acting as a bridgehead population. While the Cyprus population could have acted as a bridgehead for the French introduction or vice versa, complementary inspections found that (i) the French introduction is demographically incipient when compared to the Cyprus one and unlikely to be its origin and (ii) plant trade between Cyprus and France is non-existent while it is demonstrated to have taken place between Israel and France at plant nurseries in the vicinity of the introduced French localities (O. Blight). This favours a scenario where the Israeli population acted as an independent bridgehead for both Cypriot and French introductions. Second, the three Spanish localities displayed mtDNA and microsatellite

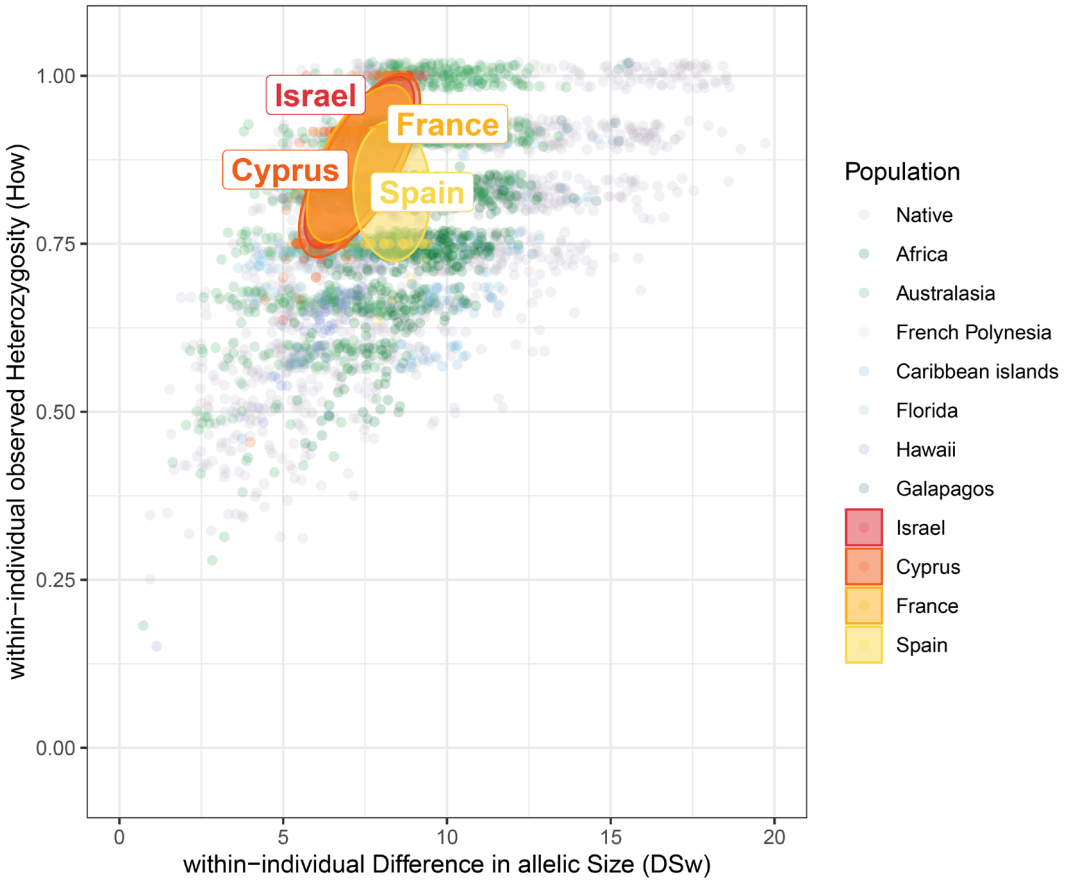

FIGURE 4 | Within-individual genetic diversity of workers of worldwide populations of *W. auropunctata*. Within-individual difference in allele size (DSw) and observed heterozygosity (Ho) are both measures of the divergence of male and female allelic pools of different populations. Ellipses regroup individuals from Mediterranean populations, assuming a multivariate t-distribution at level 80%. Colours represent populations as in Figure 1.

genotypes identical between them but quite distant from other European introductions. The mtDNA haplotype harboured by the Spanish population was found to be identical to a northeastern Argentina haplotype found in the localities of Puerto Ocampo, Chajarí and San Ignacio (Figure 1C). This result indicates that the introduction of *W. auropunctata* in Spain was due to a single long-distance introduction from northeastern Argentina, independent from the Israeli introduction.

As such, the little fire ant invasion of Europe accurately illustrates the challenges posed by the increasing interconnection between bioclimatic regions. It is crucial that biosecurity managing bodies simultaneously monitor historical sources of introduction in the native range and already introduced populations that can act as bridgeheads for additional secondary introductions. Studies of the movement of alien species worldwide, most notably using molecular datasets, now firmly establish that complex dispersal histories are the norm rather than the exception (Wilson et al. 2009). Indeed, multiple native sources, admixture events in the introduced range and secondary introductions have been demonstrated in several pest species, such as the red imported fire ant *S. invicta* (Ascunce et al. 2011), the Formosan subterranean termite *Coptotermes formosanus* (Blumenfeld et al. 2021), the bronze bug *Thaumastocoris peregrinus* (Montagu et al. 2020) or the Asian long-horned beetle *Anoplophora glabripennis* (Javal et al. 2019). In the particular case of ants, global analyses of species movement and border interceptions show that most introductions originate from secondary transport via intermediate regions, i.e., bridgehead populations (Bertelsmeier et al. 2018; Bertelsmeier and Ollier 2021). Noticeably, the lower basin of the Río de la Plata is one of the regions in the world where the largest number of highly invasive ant species, like *S. invicta*, coexist, and many source populations of unintentional introductions to other regions of the world have been traced to the main ports of this basin (Calcaterra et al. 2016).

Biosurveillance plays a critical role in addressing this issue, as many invasive ant species are discovered by accident, sometimes many years after their initial introduction (e.g., *W. auropunctata* and *S. invicta* in Europe), while the introduction of an unknown number of species remains undetected. Reducing this delay is, however, crucial to implement efficient eradication efforts and calls for significant improvements in biosurveillance (see Blight and Rabitsch 2024; Menchetti et al. 2024). In the case of *W. auropunctata*, the establishment of populations in southern China and Europe, major hubs in world trade, offers a grim perspective on the risks of future introductions. This risk is also illustrated by the fact that this documented case of bridgehead introduction is plausibly not the first in *W. auropunctata* dispersal history, as Cameroon and Hawai'i introductions probably stem from Gabon and Florida introductions, respectively (Foucaud, Orivel, et al. 2010).

4.2 | Both Long- and Short-Distance Sources of Novel Introductions Are Non-Random but Reflect Ecological Niche and Genetic Preadaptation to Invade

From an ecological standpoint, our study illustrates the importance of previous adaptation to human environments before long-distance dispersal, in line with the AIAI scenario (Hufbauer et al. 2012). In the case of *W. auropunctata*, two features are particularly salient in this respect. First, potential source populations are located in natural environments with specific disturbance regimes, e.g., the floodplains of the lower basin of the Río de la Plata for the Spanish introduction. These naturally disturbed environments may preadapt native populations to the fragmented and rapidly changing ecological conditions of anthropised areas, and ultimately allow them to reach major transport hubs. Second, its peculiar polymorphism in reproductive biology and its genetic consequences were previously shown to be instrumental in its ability to colonise human-disturbed environments, particularly regarding its thermal niche (Foucaud et al. 2013; Rey et al. 2012). European introductions respected these patterns to the line, with all populations displaying the male and female clonal system characteristic of *W. auropunctata* populations established in disturbed zones in its native range (Chifflet et al. 2018; Foucaud et al. 2009, 2013) and all of its introduced range (Foucaud, Orivel, et al. 2010). This reproduction system went also along with different female and male gene pools, resulting in an outbred workforce, again underlining the importance of this parameter in the successful establishment of *W. auropunctata* worldwide (Foucaud, Orivel, et al. 2010; Foucaud et al. 2013; Rey et al. 2012). Regarding the thermal abilities of these populations, Cypriot and French populations are simple clones of the Israeli population that showed enhanced resistance to cold and dry thermal conditions, just like its source population in Argentina (Rey et al. 2012). It is also probably the case for the Spanish introduced population, whose origin is located precisely in the climatic niche prediction corresponding to Mediterranean conditions (Coulin et al. 2019; Rey et al. 2012) and whose workers respect the pattern of outbreeding displayed by all thermally resistant populations. It is worth noting that, while heterozygosity was found to be high in introduced European populations, the difference between male and female allelic pools was slightly reduced when compared to other worldwide introductions. This may reflect a lower genetic diversity at the southern margin of the native range of *W. auropunctata*, the origin of these introductions.

4.3 | Perspectives for the Management of *W. auropunctata* in Southern Europe

Overall, the little fire ant case confirmed that anthropisation of the environment and global trade are the main triggers of the current biological invasion crisis. Invasive populations of the little fire ant may at first seem like an impossible task to handle: individuals easily concealing in globally traded vegetal products that display genetic architectures and thermal abilities tailored to the conditions of anthropised habitats, together with a theoretically perpetual heterosis maintained by its unusual clonal reproduction system. However, we argue that the situation of *W. auropunctata* might not be so dire in Europe and elsewhere. First, the actual spatial extent of Spanish and French introductions, limited to a few acres, is still compatible with their eradication management (see also Arcos et al. 2025; Pérez-Delgado et al. 2025). In contrast, the Cypriot introduction seems impossible to eradicate; however, measures to mitigate further spread and the impacts of *W. auropunctata* on native biodiversity and human health are necessary. It is therefore most urgent, both for the management of the current *W. auropunctata* situation and the prevention of other ant introductions in the EU, that administrative tools are made available and corrective actions are taken locally, following recent propositions to address policy and implementation gaps in the current EU regulation (Blight and Rabitsch 2024). As such, an eradication campaign has been started in the Toulon area of the French introduction, under the supervision of one of the authors. Besides, the dominance of *W. auropunctata* in its native range seems to be more associated with their high colonisation capacity to harsh environments (mostly anthropic) rather than its superior competitive ability (Calcaterra et al. 2016; Chifflet and Calcaterra 2024). This species showed a territorial behaviour (or local dominance) that seems to be reduced to the nesting area of the fragments that make up its supercolony, and thus its supremacy in the introduced range could only be a consequence of the exclusion of superior competitors in natural habitats (e.g., islands) or anthropic habitats (e.g., cities) (Chifflet and Calcaterra 2024).

Second, while many complex invasion histories involve secondary genetic admixture in the introduced range and increasing of available genetic diversity (Kolbe et al. 2004; Lavergne and Molofsky 2007; Yainna et al. 2022), the particular biology of the little fire ant renders this future scenario highly unlikely. Indeed, clonal populations do not mix and rather fiercely try to eliminate each other (Foucaud 2007; Orivel et al. 2009), with only a possible exchange of genetic material in the form of 'captured' males. These events are rare because males are scarce and actively kept in nests by workers. While the genetic sequestration of males can be an issue in its native range, where genetic diversity is high (Foucaud 2007), the extreme depletion of genetic diversity seen in introduced populations (only one haploid male genome per population) severely limits any risk posed by genetic admixture.

Third, our large sampling of both male and female genotypes allowed the detection of rare events of sexual production of males and queens, in all European introductions. Though these events may seem insignificant at first, they set in motion the gradual mixing of the extremely limited male and female genetic diversity found in introduced populations (only one haploid male genome and one diploid female genome), with new queens progressively harbouring male alleles. This phenomenon, which was first detected in ancient introduction areas such as New Caledonia (Foucaud et al. 2006), was also uncovered here in Spain. Theoretically, this could lead to complete genetic mixing of male and female gene pools, the loss of heterosis and extreme inbreeding depression in workers and a possible breakdown of the invasion process. Whether this could happen in the future of European introductions may be better forecasted by investigating in detail what happened relative to genetic mixing of male and female gene pools to earlier introductions, and notably to the New Caledonian introduction during the last 20 years.

4.4 | Conclusions

The recent introductions of *W. auropunctata* in Europe therefore illustrate some of the main forces at play in biological invasions: anthropisation leading to adaptation and global movements mixing long-distance dispersal from the native range and shorter secondary transfers from bridgehead populations. While the little fire ant seems to possess an outstanding advantage with its unusual clonal reproduction system, it may also constitute its main weakness and provoke its decline in the longer term. However, given the risks posed by both this species and other invasive ant species for European environments and economies, direct handling of these introductions when still possible is urgently needed. This should be accompanied by the development and enforcement of targeted management strategies and regulatory frameworks to prevent and/or mitigate future ecological impacts and economic costs.

Author Contributions

Conceptualization: A.L., B.F. and J.F. Data curation: A.L., G.J.K. and J.F. Data analyses and methodology: B.F., G.J.K. and J.F. Funding acquisition: J.D. and J.F. Investigation: A.L. and L.B. M.M. Resources: H.J., J.D., L.A.C., L.C., M.M., O.B. and X.E. Writing – original draft preparation: A.L., B.F., G.J.K., J.D., J.F., L.A.C. and M.M. Writing – review and editing: All contributors read the MS and provided edits.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data and code generated during this study have been deposited in the INRAE dataverse under the doi: https://doi.org/10.57745/XZANYY.

Peer Review

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.