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ORIGINAL PAPER

Invasion genetics of the Asian hornet *Vespa velutina nigrithorax* in Southern Europe

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Abstract In 2004, *Vespa velutina* was first seen in France. Since then, this fierce honey bee predator spread across many countries, giving rise to one of the most phenomenal insect invasions in Europe. An early study in France showed a genetically depauperate population, originating from a single multi-mated queen introduced from China. Here, we further unveil *V. velutina* invasion genetics in Europe by surveying the Iberian and Italian peninsulas using cytonuclear markers. Our results show that the French population acted as the colonists' source in Spain, Portugal and Italy, leading to rejecting the hypothesis of multiple introductions from the native range. While Spain and Italy were colonized predominantly by leading-edge expansions from the French core population, in Portugal the invasion started from long-distance jump. Both processes were accompanied by a significant reduction in genetic diversity, with stronger losses for Portugal (Ar = 17.4%; uHe = 42.3%) than for Spain (Ar = 9.0%; uHe = 20.6%) or Italy (Ar = 16.3%; uHe = 26.8%). Signatures of differentiation and population structure, associated to the founding event in Portugal, enabled detection of secondary contact between the front derived from the primary propagule introduced in France and the front derived from the secondary propagule introduced in Portugal. Detection of first-generation migrants in the three countries suggests continuous gene flow that is bringing in new alleles, and this effect is stronger in Portugal, as reflected by a 20.3% increase in allelic richness. Overall, this study provides further insights into the invasion genetics of *V. velutina* in Europe, which can

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aid developing strategies to manage this major threat to beekeeping.

Keywords Yellow-legged hornet · Alien species · Biological invasion · Genetic diversity · Microsatellites · mtDNA

Introduction

In the past century, numerous insects have successfully colonized new territories around the globe (reviewed in Beggs et al. 2011; Elder and Bell 1998; Kenis et al. 2009; Paine and Millar 2002). Yet, of special concern are social Hymenoptera, with six species in the top-100 IUCN list of the worst alien invaders (http://go.nature.com/qa9z1g). The Asian hornet, *Vespa velutina nigrithorax* de Buysson (hereafter referred to as *V. velutina*), has not yet earned a place in the IUCN infamous list. However, the phenomenal spread across western Europe, adversely affecting biodiversity, ecosystem services and even human health, led to its inclusion in the European Union's (EU) black-list of invasive alien species (EU regulation No. 1143/2014).

V. velutina shares with many other social Hymenoptera a number of unique biological and life history traits that enhance the probability of successful establishment and subsequent spread in new territories (reviewed in Moller 1996). Polyandry is one such trait and *V. velutina* is one of the rare polyandrous species in Vespidae (Arca et al. 2015). Depending on the mating frequency in the original habitat, a few (or even a single) founder queens may carry in their spermatheca a representative sample of the genetic diversity of the source population (Ding et al. 2017; Mikheyev et al. 2009; Schmid-Hempel et al. 2007). This trait greatly aids the early stage of propagule establishment by lessening the effects of genetic drift and increasing the likelihood of fast adaptive response to the new selective regimes in the recipient environment (Estoup et al. 2016). Once established, a suit of other traits provides additional adaptive benefits, facilitating spread across the novel habitats, including (i) exceptional dispersal abilities, (ii) high reproductive efficiency, (iii) broad diets and habitat ranges, (iv) effective predator defences, and (v) ability of buffering against environmental changes (reviewed in Beggs et al. 2011; Moller 1996).

While over 30 species of Vespidae were successfully introduced around the world in the last decades (Beggs et al. 2011), Europe was free of these invaders until arrival of a single mated queen of *V. velutina* (Arca et al. 2015) to the French district of Lot-et-Garonne in 2004 (Arca et al. 2015; Haxaire et al. 2006; Villemant et al. 2006). *V. velutina* has since expanded across most of France and made its way into nine neighbouring countries. It took six years to the first incursion of *V. velutina* outside of France. In 2010, the first specimen was captured in the Spanish locality of Amaiur, 180 km south of the introduction point in Nérac, Lot-et-Garonne (López et al. 2011). However, soon after Spain, several other countries registered the entry of *V. velutina* in their territories, including Belgium (Rome et al. 2013) and Portugal (Grosso-Silva and Maia 2012) in 2011, Italy in 2012 (Demichelis et al. 2014), Germany in 2014 (Witt 2015), Great Britain (Budge et al. 2017) and the Netherlands in 2016 (Smit et al. 2018), and Switzerland in 2017 (Poidatz et al. 2018). The recent detection in Hamburg, Germany, far from the leading edge in the Netherlands, represents the northernmost point in Europe (Husemann et al. 2020). While the geographical limits in Europe are yet to be reached (Barbet-Massin et al. 2013, 2018; Bessa et al. 2016; Keeling et al. 2017; Robinet et al. 2017), currently, *V. velutina* occupies a large continuous territory in the western countries, from Portugal to the Netherlands and across the English Channel in Great Britain, and it is spreading eastwards into Italy (see the dynamic map in http://frelonasiatique.mnhn.fr/).

The spread of *V. velutina* in Europe is causing economic and ecological losses as well as human fatalities (Monceau et al. 2014). While the yearly costs of nest destruction (the main control measure) have exceeded €29 million in the United Kingdom, France and Italy (Barbet-Massin et al. 2020), the damage to wild ecosystems, agriculture and beekeeping is likely much larger but yet to be fully appraised. *V. velutina* is disrupting invaded ecosystems by competing with the native entomofauna (Monceau et al. 2015) and, primarily, by interfering with pollination of wild plants and crops as it feeds upon many pollinator insects (Monceau et al. 2013a), most notably the western honey bee (*Apis mellifera*). In Autumn, when the hornet's nest size and protein intake by the sexual

brood reach their maxima (Monceau et al. 2013b), hundreds of hornets hover at the front of beehives disrupting foraging, a critical activity at this time of the year as colonies are preparing for winter. This disturbance can lead to depletion of food stores and to depopulation of beehives, enhancing the likelihood of winter colony mortality (Requier et al. 2019).

Spain, Portugal, and Italy are home to 5.4 million colonies, representing 29.4% (as in 2019) of the honey bee stock and 22.3% (as in 2018) of the honey production in the European Union. In a scenario of growing colony losses (Neumann and Carreck 2010), sustaining these figures implies additional expenses with apiary management and thereby a heavier financial burden to the beekeeping industry. The drivers of colony losses are complex and interacting and include a diverse repertoire of alien pests, such as the ectoparasitic mite *Varroa destructor*, and its vectored viruses, the beetle *Aethina tumida,* the microsporidia *Nosema ceranae*, and now *V. velutina* (Laurino et al. 2020; Martín-Hernández et al. 2018; Mutinelli et al. 2014; Steinhauer et al. 2018). Unfortunately, the early attempts to halt establishment and spread of *V. velutina* in France were insufficient and inefficient. Also inefficient were the Pyrenees and the Alps in deterring its natural range expansion into the Iberian and Italian peninsulas (Bertolino et al. 2016; Goldarazena et al. 2015; López et al. 2011). On the other hand, the arrival of *V. velutina* to Viana do Castelo (Minho, Portugal), in 2011, and to A Mariña (Galiza, Spain), in 2012, over 620 and 480 km apart, respectively, from the expanding front in Navarra and Basque Country (Grosso-Silva and Maia 2012; López et al. 2011), likely resulted from a human-mediated jump within Europe, although the hypothesis of independent introductions from the native range cannot be overlooked.

V. velutina has established itself successfully in Iberia, where it occupies a continuous area from Catalonia (Mediterranean) to Lisbon (Atlantic), and it is currently expanding in the Italian peninsula along the western coast (Bertolino et al. 2016; Porporato et al. 2014). The mean expansion rate ranges from 18.3 in Italy (Bertolino et al. 2016) to 37.7 km year^{-1} in Portugal (Carvalho et al. 2020), suggesting that *V. velutina* is spreading at a slower pace in these territories than in France, where an invasion speed of up to 82 km year^{-1} was documented (Robinet et al. 2017). Despite the surveillance and control actions enforced by Reg. (EU) No. 1143/2014 to the EU member states, populations have rapidly grown to large numbers, as reflected by nest densities of 3.5 nests km^{-2} in Italy (Bertolino et al. 2016) and 5.4 nests km^{-2} in Portugal (Carvalho et al. 2020).

While there is a wealth of studies on different aspects of the biology, ecology and management of *V. velutina* in Europe (reviewed in Laurino et al. 2020; Monceau et al. 2014; Turchi and Derijard 2018), there are only a few addressing the genetic aspects of the invasion (Arca et al. 2015; Budge et al. 2017; Granato et al. 2019; Jones et al. 2020). In these studies, mitochondrial and nuclear DNA data are consistent with a founder event starting from a single mated queen (Arca et al. 2015), whose descendants reached Italy by natural expansion (Bertolino et al. 2016; Granato et al. 2019) and Great Britain by human-mediated jump (Budge et al. 2017). Here, we expanded current knowledge on the invasion genetics of *V. velutina* in Europe by reporting on the results of a mitochondrial (cytochrome C oxidase I) and nuclear (microsatellites) DNA screening of the invading populations in the Iberian and Italian peninsulas. Our aim was (i) to test whether the invading populations derive from the introduced propagule in France or from the native range in Southeast Asia, (ii) to determine the extent of genetic diversity impoverishment relative to the French and Asian populations, and (iii) to infer genetic structure.

Materials and methods

Sampling and DNA extraction

A total of 246 adult *V. velutina* females were collected in Spain (N = 45), Portugal (N = 190) and Italy (N = 11) from founding nests, secondary nests, and from traps placed in apiaries between March and December of 2016 and 2017 (see sampling details in Table S1). Apiaries were selected to be at least 3 km far apart to avoid over-representing the queen genotype by sampling multiple individuals from the same colony. Samples were placed in absolute ethanol and stored at − 20 °C until molecular analysis. Total DNA was extracted from the thorax of the 246 individuals, each representing a single colony, using the Ron's Tissue DNA Mini Kit (®Bioron), according to the protocol provided by the manufacturer.

Mitochondrial DNA sequencing and data analysis

A sub-sample of 13 individuals from Spain, 22 from Portugal, and 3 from Italy (Table S1), selected in each country to cover a wide geographical range, was amplified at the mitochondrial DNA (mtDNA) 642 bp barcode region of the Cytochrome C Oxidase Subunit I (COI) gene, using the universal primers LCO-1490 and HCO-2198 (Folmer et al. 1994). Polymerase chain reaction (PCR) amplification was carried out to a final volume of 25 μL which contained 0.2 mM of dNTPs (Promega), 2 mM of $MgCl_2$, 1X *Taq* DNA polymerase buffer, 0.2 μM of each primer, 0.625U of GoTaq®–Flexi DNA polymerase (Promega) and 10 ng of DNA. PCR was performed in a T100 Thermal Cycler (BioRad™) using an initial denaturation step of 94°C for 5 min, followed by 35 cycles of 30 s at 94 °C, 45 s at 50 °C and 1 min at 72 °C, and a final extension of 10 min at 72 °C. PCR products were sent to STABVIDA Inc. (Portugal) for direct Sanger sequencing in both directions. DNA sequences were checked manually for base calling and aligned using MEGA 7.0.26 (Kumar et al. 2016).

The 38 sequences were aligned manually with 23 sequences of *V. velutina* downloaded from GenBank using MEGA. To infer the phylogenetic relationships among the 61 samples, the maximum parsimony method was implemented in MEGA. The tree was obtained using the subtree-pruning-regrafting (SPR) algorithm (Nei and Kumar 2000) with search level zero, in which the initial trees were obtained by the random addition of sequences (10 replicates). The resulting tree was rooted with *Vespa bicolor* (accession number KT257112), *Vespa vivax* (KT257116) and *Vespa affinis* (KJ147242).

Microsatellite genotyping and data analysis

The 246 individuals were genotyped for 21 microsatellite loci using the primers developed by others (Arca et al. 2012; Daly et al. 2002; Hasegawa and Takahashi 2002). The forward primers were labelled with 6-FAM, HEX, Atto 550 or Atto 565 fluorescent dyes and the PCR amplifications were performed in three multiplexes (Table S2). The PCR reactions contained 1 μL of DNA (10 ng/μL), 5 μL of Multiplex PCR kit (Qiagen), and 1 μL of a 2 μM primer mix, in a 10 μL final volume. Reactions were performed in a T100 Thermal Cycler (BioRad™) using a temperature profile consisting of an initial denaturation step at 95 °C for 15 min followed by 40 cycles of 95 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min, and a final extension of 60 °C for 60 min. Fragment analysis was performed on an ABI PRISM 3730 × 1 automated Genetic Analyzer (Applied Biosystems, Foster, California, USA), using GeneScan 500LIZ® as the internal size standard, at STABVIDA Inc. (Portugal). The lengths of the fragments were determined using GENEMAPPER 3.7 (Applied Biosystems) and each allele was checked manually. To have an estimate of the amplification error rate, 30 randomly selected samples were genotyped twice and analysed with GIMLET 1.3.3 (Valière 2002). Further scoring errors due to null alleles and allelic dropout were assessed in MICRO-CHECKER v.2.2.3 (Van Oosterhout et al. 2004).

The microsatellite dataset generated for the 246 individuals was merged with an existing dataset containing genotypes of 162 individuals collected in the introduced (France = 83) and native ranges (Chinese provinces of Yunnan = 20 and Zhejiang/Jiangsu = 30, Indonesia = 21, and Vietnam = 8) of *V. velutina* (Arca et al. 2015). To enable dataset merging, allele scores of each microsatellite locus were harmonized between laboratories by genotyping 10 DNA samples used by Arca et al. (2015).

Allele frequencies, observed number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He) and unbiased expected heterozygosity (uHe) were computed per locus and country using GENALEX 6.5 (Peakall and Smouse 2012) whereas allelic richness (Ar) was computed using the package HIERFSTAT (Goudet 2005) in RStudio v1.2.5033 (RStudio Team, 2015). The diversity metrics uHe, calculated using the standard approach of Nei (1978), and Ar, calculated using the rarefaction method (El Mousadik and Petit 1996), are adjusted for unequal sample size. Differences in Na, Ar, and uHe between population samples were assessed by the Wilcoxon's signed-rank test, also implemented in RStudio. Genetic differentiation among populations from the invaded and native ranges was estimated by pairwise F_{ST} values computed in ARLEQUIN 3.5.2.2 (Excoffier et al. 2005), using 10 000 random permutations to assess significance. Whenever needed, statistical significance levels were adjusted for multiple comparisons using the sequential Bonferroni procedure (Rice 1989).

To identify the most likely source of invaders in Spain, Portugal and Italy, the 246 individuals were assigned to the Asian and French reference populations from Arca et al. (2015) using the Bayesian-based assignment test (Rannala and Mountain 1997) and the Monte-Carlo resampling algorithm of Paetkau et al. (2004), with 10 000 replicates, in GENECLASS 2 (Piry et al. 2004). The assignment test estimates the likelihood that an individual belongs to a population and assigns the population with the highest likelihood as the origin of the sampled individual. Considering that not all potential source populations have been sampled, first-generation migrants in each of the three invaded countries were identified using L_home as the statistical criterion for likelihood estimation (Piry et al. 2004). A threshold of 0.01 was set for type I error.

To assess genetic relationships among individuals and whether there is genetic structure in Europe, a model-free principal coordinate analysis (PCoA) was run in GENALEX 6.5 (Peakall and Smouse 2012), using the option Genetic Distance (Codom-Genotypic) for codominant data, and a model-based Bayesian clustering analysis was run in STRUCTURE 2.3.3 (Pritchard et al. 2000) using the merged dataset (N = 408). The STRUCTURE analysis was performed using the admixture ancestry and correlated allele frequency models with the unsupervised option to estimate the membership proportion (Q-value) to each ancestral cluster (K) for each individual. The program was set up for 750 000 Monte Carlo Markov chain iterations, after an initial burn-in of 250 000, and run for 20 independent replicates for each K (from 1 to 5). Q-plots were post-processed online with CLUMPAK (Kopelman et al. 2015).

Results

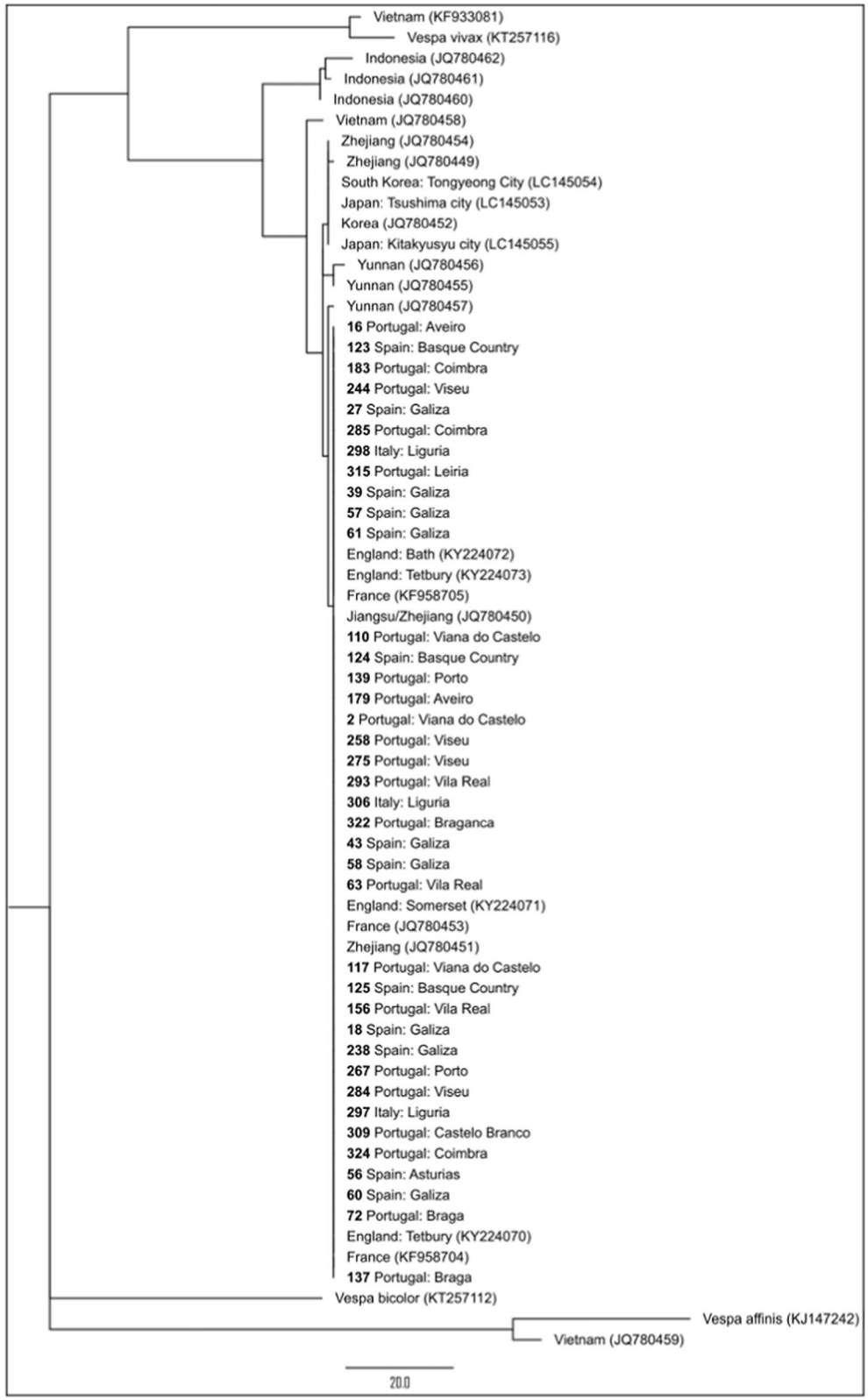
Mitochondrial diversity

Mitochondrial DNA (mtDNA) variation was analysed using sequence data of the 642 bp COI barcode region obtained from 38 individuals from Spain, Portugal, and Italy. A single haplotype, named by Arca et al. (2015) as F, was identified in these individuals, as shown in the maximum parsimony phylogenetic tree (Fig. 1). The European samples were placed in the same clade, together with two samples (JQ780450 and JQ780451) from Zhejiang, and with a close phylogenetic proximity to a sequence from Yunnan (JQ780457), further supporting the claim of eastern China as the origin of *V. velutina* propagules in Europe (Arca et al. 2015).

Microsatellite diversity

A total of 21 microsatellite loci were screened in the 246 individuals from Spain, Portugal, and Italy. Five of these loci were initially excluded from further analysis due to inconsistent amplification. The remaining 16 loci were successfully amplified with virtually no missing data (see genotypes in Table S1 and missing data in Table S2). According to the tests run in GIMLET and MICRO-CHECKER, there were barely any signs of allelic dropout (Tables S3 and S4). In contrast, null alleles were detected for all loci, but VMA8, and in all countries, but Italy (Table S4). Yet, the 15 loci did not overlap within and between the European and the Asian populations, suggesting that the loci themselves are not faulty. Instead, it is possible that the null alleles are an artifact from joining samples (collected throughout wide geographical areas and in different years) by country, causing a Wahlund effect. Support for this hypothesis comes from the geographically-narrow sample collected in Italy and from the sub-sample collected in 2016 in Viana do Castelo (the place of *V. velutina* introduction in Portugal) with no null alleles (Table S4). Accordingly, we did not discard any locus and performed the ensuing analysis using the 16 loci.

The mean (± SE) number of alleles (Na, Ne), allelic richness (Ar), and heterozygosities (Ho, uHe) computed for each country of the invaded and native ranges in Europe and Asia are shown in Table 1 (but see Table S5 for allele frequencies and metrics per locus and population). As expected for a founder event, the genetic diversity of *V. velutina* was dramatically reduced following its introduction in Europe. The 16 microsatellite loci had a total of 190 different alleles in the eight population samples analysed, of which 186 were present in Asia and 65 in Europe. While the number of private alleles varied between five (Vietnam) and 20 (Zhejiang/Jiangsu) in the native range, in Europe there was only one private allele detected in the Spanish population. In Asia, all loci were polymorphic whereas in Europe two loci were monomorphic (R1-77 in Spain, Portugal, and Italy; R1-80 only in Portugal). Significantly fewer (P-


Vietnam (KF933081)
Vespa vivax (KT257116)
Indonesia (JQ780462)
Indonesia (JQ780461)
Indonesia (JQ780460)
Vietnam (JQ780458)
Zhejiang (JQ780454)
Zhejiang (JQ780449)
South Korea: Tongyeong City (LC145054)
Japan: Tsushima city (LC145053)
Korea (JQ780452)
Japan: Kitakyusyu city (LC145055)
Yunnan (JQ780456)
Yunnan (JQ780455)
Yunnan (JQ780457)
16 Portugal: Aveiro
123 Spain: Basque Country
183 Portugal: Coimbra
244 Portugal: Viseu
27 Spain: Galiza
285 Portugal: Coimbra
298 Italy: Liguria
315 Portugal: Leiria
39 Spain: Galiza
57 Spain: Galiza
61 Spain: Galiza
England: Bath (KY224072)
England: Tetbury (KY224073)
France (KF958705)
Jiangsu/Zhejiang (JQ780450)
110 Portugal: Viana do Castelo
124 Spain: Basque Country
139 Portugal: Porto
179 Portugal: Aveiro
2 Portugal: Viana do Castelo
258 Portugal: Viseu
275 Portugal: Viseu
293 Portugal: Vila Real
306 Italy: Liguria
322 Portugal: Braganca
43 Spain: Galiza
58 Spain: Galiza
63 Portugal: Vila Real
England: Somerset (KY224071)
France (JQ780453)
Zhejiang (JQ780451)
117 Portugal: Viana do Castelo
125 Spain: Basque Country
156 Portugal: Vila Real
18 Spain: Galiza
238 Spain: Galiza
267 Portugal: Porto
284 Portugal: Viseu
297 Italy: Liguria
309 Portugal: Castelo Branco
324 Portugal: Coimbra
56 Spain: Asturias
60 Spain: Galiza
72 Portugal: Braga
England: Tetbury (KY224070)
France (KF958704)
137 Portugal: Braga
Vespa bicolor (KT257112)
Vespa affinis (KJ147242)
Vietnam (JQ780459)
20.0

◀**Fig. 1** Maximum Parsimony (MP) phylogenetic tree for the 38 V. *velutina* individuals from Spain, Portugal, and Italy sequenced in this study. The MP tree was generated from 64 COI sequences with a length of 598 bp (256 nucleotide positions were eliminated because they contained gaps or missing data). The MP was obtained with a tree length of 196. MP inferred a consistency index of 0.844, a retention index of 0.912, and a composite index of 0.770 for parsimony-informative sites. The tree is drawn to scale, with branch lengths calculated using the average pathway method (Nei and Kumar 2000) and are in the units of the number of changes over the whole 598-bp COI sequence. The analysis included 64 additional sequences from individuals of the invaded range in Europe, South Korea, and Japan, and from the native range in China (Yunnan, Jiangsu/Zhejiang), Vietnam and Indonesia

value $\leq$ 0.0036, Wilcoxon's signed-rank test) alleles were detected in Italy (2.062 $\pm$ 0.170), Portugal (2.312 $\pm$ 0.198) and Spain (2.438 $\pm$ 0.182) than in France (3.938 $\pm$ 0.452) or in the Chinese province of Zhejiang/Jiangsu (7.062 $\pm$ 1.039).

Likewise, mean allelic richness and unbiased expected heterozygosity (measures adjusted for unequal sample size) were significantly lower (*P*-value $\leq$ 0.0171, Wilcoxon's signed-rank test) in any of the three more recently invaded countries (Portugal: Ar = 2.985, uHe = 0.311; Italy: Ar = 3.024, uHe = 0.395; Spain: Ar = 3.291, uHe = 0.428) than in France (Ar = 3.615, uHe = 0.538) or Asia (4.089 $\leq$ Ar $\leq$ 5.365; 0.588 $\leq$ uHe $\leq$ 0.788), further intensifying drift effects of the introduction bottleneck. Of the three countries, Portugal suffered the heaviest loss of genetic diversity associated to the founder event, with Ar and uHe reaching 17.4% and 42.3%, or 29.6% and 53.9%, respectively, depending on whether France or Zhejiang/Jiangsu were used as the propagule source (Fig. 2). Lower reductions in these diversity metrics were observed for Spain (Ar = 9.0%; uHe = 20.6%) and Italy (Ar = 16.3%; uHe = 26.8%).

Source of invaders

The most likely origin of *V. velutina* invasion in Spain, Portugal, and Italy was identified by analysing all pairwise F_{ST} values and mean probability assignments using the Asian and French populations as potential sources (Table 2). A high level of genetic differentiation was observed for the intercontinental comparisons (mean $\pm$ SE; 0.362 $\pm$ 0.031), being the lowest F_{ST} values obtained with the Chinese province of

Table 1 Observed number of alleles (Na), effective number of alleles (Ne), number of private alleles (NPa), allelic richness (Ar), observed heterozygosity (Ho), and unbiased expected heterozygosity (uHe) across loci and population (mean $\pm$ SE)

	SP	PT	IT	FR	VT	IND	YN	ZJ
Na	2.438 $\pm$ 0.182	2.313 $\pm$ 0.198	2.063 $\pm$ 0.170	3.938 $\pm$ 0.452	3.750 $\pm$ 0.403	5.563 $\pm$ 0.532	7.250 $\pm$ 0.595	7.063 $\pm$ 1.039
Ne	1.847 $\pm$ 0.118	1.613 $\pm$ 0.136	1.718 $\pm$ 0.103	2.328 $\pm$ 0.155	2.707 $\pm$ 0.348	3.583 $\pm$ 0.403	4.973 $\pm$ 0.438	4.241 $\pm$ 0.607
NPa[a]	1	0	0	0	5	19	15	20
Ar	3.291	2.985	3.024	3.615	4.089	4.950	5.365	4.242[b]
Ho	0.342 $\pm$ 0.042	0.302 $\pm$ 0.059	0.369 $\pm$ 0.055	0.459 $\pm$ 0.042	0.523 $\pm$ 0.066	0.461 $\pm$ 0.056	0.725 $\pm$ 0.044	0.656 $\pm$ 0.071
uHe	0.428 $\pm$ 0.040	0.311 $\pm$ 0.058	0.394 $\pm$ 0.049	0.539 $\pm$ 0.038	0.588 $\pm$ 0.049	0.679 $\pm$ 0.049	0.789 $\pm$ 0.026	0.674 $\pm$ 0.071

[a]Private alleles with a frequency > 0.01. [b]Allelic richness was estimated from only 13 loci, given the high rate of missing data for the three loci discarded. Country codes: SP – Spain, PT – Portugal, IT – Italy, FR – France, VT – Vietnam, IND – Indonesia, YN – Yunnan (China), and ZJ—Zhejiang/Jiangsu (China)

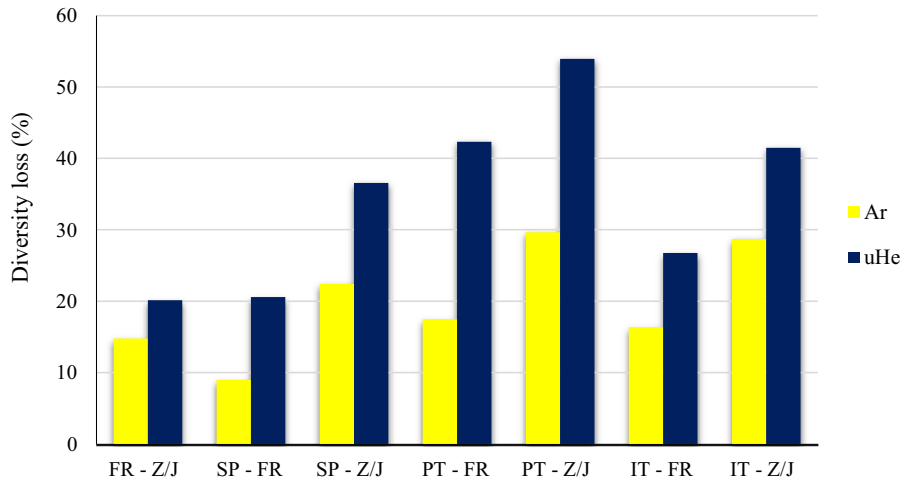


Fig. 2 Diversity loss, measured by allelic richness (Ar) and unbiased heterozygosity (uHe), for the populations of France (FR), Spain (SP), Portugal (PT) and Italy (IT), relative to Zhejiang/Jiangsu (Z/J) or France. Loss uHe = 1 – (uHe_i/ uHe_s) and Loss Ar = 1 – (Ar_i/Ar_s), with the values calculated across loci in the invasive population (i) and in the putative source region (s)

Table 2 Multi-locus estimates of F_{ST} (below diagonal) and assignment probability (above diagonal; mean ± SE) between populations of the invaded and native ranges

Country	SP	PT	IT	FR	VT	IND	YN	Z/J
SP				0.735 ± 0.031	0.000 ± 0.000	5.33e-05 ± 1.08e-05	0.015 ± 0.002	0.329 ± 0.026
PT	*0.070*			0.695 ± 0.013	0.000 ± 0.000	7.26e-05 ± 3.94e-06	0.015 ± 0.001	0.434 ± 0.010
IT	*0.193*	*0.325*		0.668 ± 0.058	0.000 ± 0.000	1.55e-04 ± 2.85e-05	0.012 ± 0.002	0.430 ± 0.036
FR	*0.074*	*0.210*	*0.104*					
VT	*0.434*	*0.552*	*0.424*	*0.340*				
IND	*0.355*	*0.481*	*0.320*	*0.251*	*0.269*			
YN	*0.295*	*0.448*	*0.260*	*0.212*	*0.179*	*0.115*		
Z/J	*0.217*	*0.352*	*0.200*	*0.117*	*0.143*	*0.150*	0.000	

F_{ST} values in italics are significantly different from zero (all P-value < 0.000). See Table S6 for individual assignment probabilities and scores. Country codes: SP – Spain, PT – Portugal, IT – Italy, FR – France, VT – Vietnam, IND – Indonesia, YN – Yunnan (China), and Z/J—Zhejiang/Jiangsu (China)

Zhejiang/Jiangsu (0.256 ± 0.048). A reduction in divergence was observed when pairwise comparisons were made with France (0.129 ± 0.041), with Spain exhibiting the lowest (0.074) and Portugal the highest (0.210) F_{ST} values.

The mean assignment probabilities for the three invasive populations are shown in Table 2. Every single individual sampled in Spain, Portugal, and Italy was assigned to France (Table S6), with a score of 100% and assignment probabilities varying between 0.277 and 0.999 for Spain (0.735 ± 0.031), 0.262 and 0.979 for Portugal (0.695 ± 0.013), and 0.410 and 0.961 for Italy (0.668 ± 0.058). These metrics are considerably higher than those obtained for Zhejiang/Jiangsu, the second most likely source of origin, with a mean score of 0% and mean assignment probabilities varying between 0.329 ± 0.026 for Spain and 0.434 ± 0.010 for Portugal (Tables 2 and S6). Altogether, the results from F_{ST} and assignment tests suggest that France acted as the most likely source of the colonists that invaded both the Iberian and Italian peninsulas.

Population structure

Genetic relationships of all individuals from Europe and Asia were first investigated using the model-free principal coordinate analysis (PCoA). Individuals from Portugal and Asia fell at the two extremes of the spectrum defined by coordinate 1 axis, which

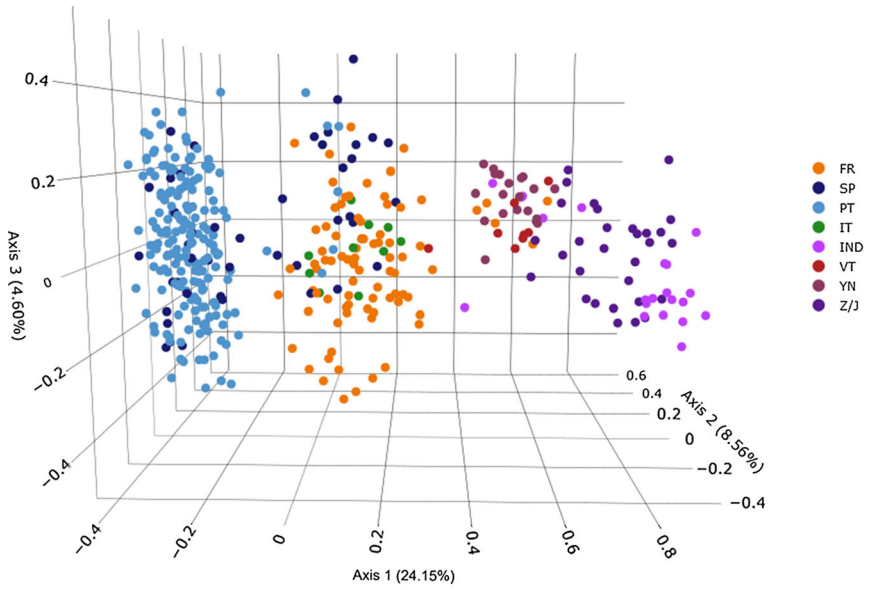


Fig. 3 Principal coordinate analysis (PCoA). Axis one, two, and three account for 24.15%, 8.56%, and 4.60% of the variance, respectively. Country codes: FR – France, SP – Spain, PT – Portugal, IT – Italy, IND – Indonesia, VT – Vietnam, and YN – Yunnan (China), and Z/J—Zhejiang/Jiangsu (China)

explains 24.2% of the total variation (Fig. 3). Most individuals from Portugal (96.3%) and Spain (53.3%) formed a tight cluster, which is placed in the three-dimensional space further apart from the Asian cluster than from the cluster that joins all individuals from France and Italy (Fig. 3).

A pattern of genetic heterogeneity in the invaded range in Europe was also captured by the Bayesian model-based approach implemented in STRUCTURE (Fig. 4). At K = 2, there was a clear separation between invasive and native populations. Further partitioning of variation (K = 3) unveils the existence of genetic structure in Europe, with the individuals from France (mean Q-value ± SE; 0.958 ± 0.007; Table S7) and Italy (0.975 ± 0.008) placed together in one cluster, coloured by orange in the graphical representation of structure (Fig. 4). In contrast, individuals from Portugal were placed together in the blue cluster (0.958 ± 0.013), except for six individuals that exhibited a clear ancestry in the orange cluster (Q-value ≥ 0.934). Individuals from Spain displayed mean intermediate Q-values (0.528 ± 0.071), with 19 individuals assigned to the orange cluster (0.927 ≤ Q-value ≤ 0.995) and 23 to the blue cluster (0.910 ≤ Q-value ≤ 0.996). The six Portuguese individuals assigned to the orange cluster were also detected by GENECLASS as first-generation migrants (*P*-value ≤ 0.0019; Table S8) with a putative origin in Spain (7.936 ≤ − log(L) ≤ 10.547). GENECLASS also identified two migrants in Spain (*P*-value ≤ 0.0006; 9.998 ≤ − log(L) ≤ 11.425) and one in Italy (*P*-value ≤ 0.0000; -log(L) ≤ 8.965), but they were both originated from the French population (Fig. 4). Interestingly, when the first-generation migrants were removed from the population samples, there was a decrease in diversity in Spain, Portugal, and Italy, which was more pronounced for allelic than for heterozygosity measures, with Ar varying between 20.3% (Portugal) and 13.2% (Italy) and uHe varying between 3.3% (Portugal) and 2.0% (Spain. See Fig. 5 and Table S9).

Discussion

The mitochondrial and nuclear markers concurrently employed herein suggest that the invasions of *V. velutina* into the Iberian and Italian peninsulas stemmed from the particularly successful population of France, rather than from multiple independent introductions from the native range. Convincing evidence comes from the unique mitochondrial

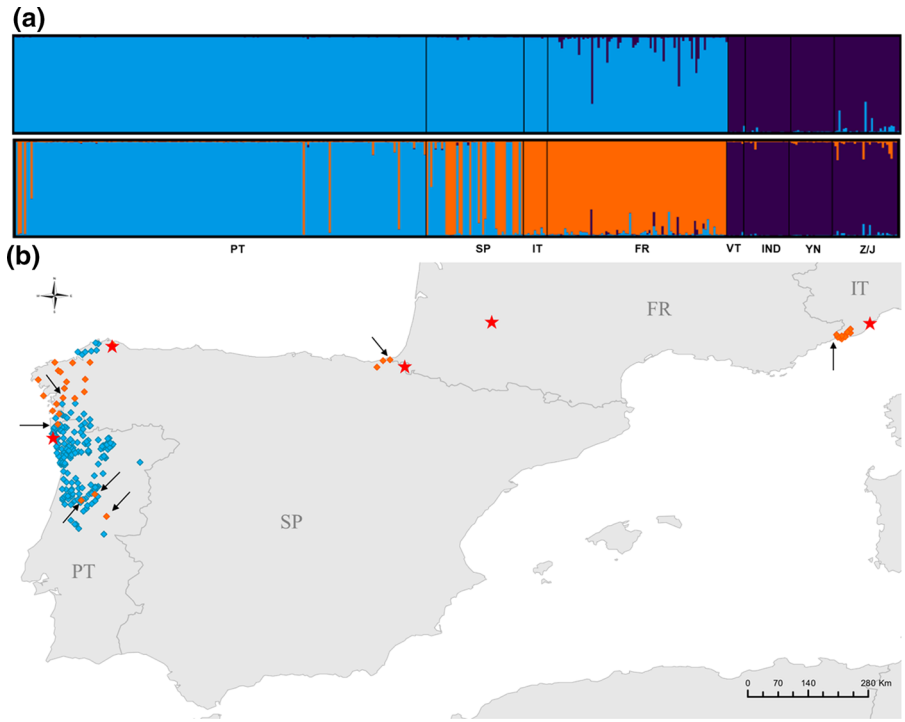


Fig. 4 **a** STRUCTURE plots showing in the Y-axis the membership partitioning (Q-values) of *V. velutina* variation into two (top plot) and three (bottom plot) K clusters (see Table S7 for Q-values) for each individual of the invading and native ranges (X-axis). Each individual is represented by a vertical bar. Vertical black lines separate individuals from different populations. Country codes: PT – Portugal, SP – Spain, IT – Italy, FR – France, VT – Vietnam, IND – Indonesia, YN – Yunnan (China), and Z/J – Zhejiang/Jiangsu (China). **b** Geographical distribution of the individuals sampled in SP, PT, and IT. The orange dots correspond to the individuals with Q-values $\geq$ 0.90 into the French cluster and the blue dots with Q-values $\geq$ 0.85 into the Portuguese cluster. The arrows point to first-generation migrants, identified using L-home as the statistical criterion for likelihood estimation, with a threshold of 0.01 for type I error (see Table S8 for details). The red stars indicate the location of the first sightings of *V. velutina* in Nérac (Lot-et-Garonne, FR) in 2004, Amaiur (Navarra, SP) in 2010, Viana do Castelo (PT) in 2011, A Mariña (Galiza, SP) in 2012, and Loano (IT) in 2012. The arrow nearby Viana do Castelo points to three overlapping individuals

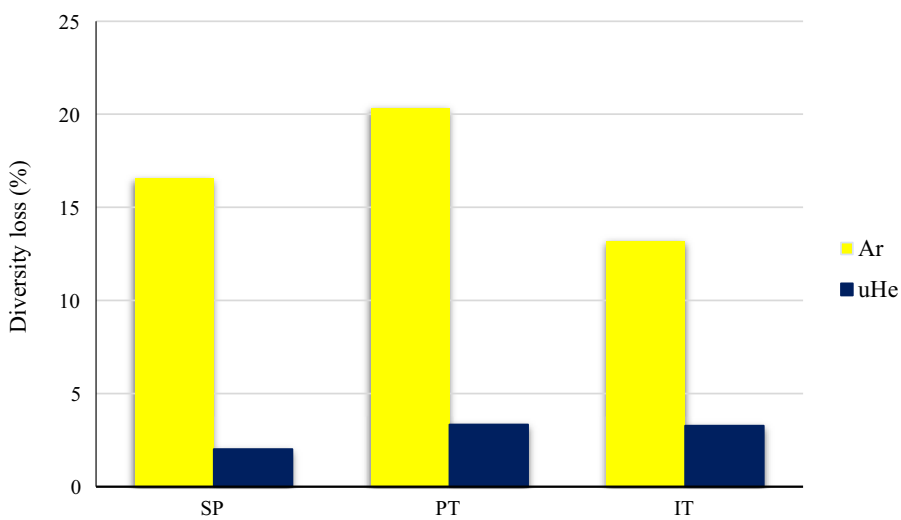


Fig. 5 Diversity loss in Spain (SP), Portugal (PT) and Italy (IT), when first-generation migrants are removed from each population sample. Loss uHe = 1 – (uHe_d/ uHe_r) and Loss Ar = 1 – (Ar_d/Ar_r), being allelic richness (Ar) and unbiased heterozygosity (uHe) calculated across loci for each sample with first-generation migrants discarded from the dataset (d) and retained in the dataset (r)

haplotype identified in the area under study, congruent with other mitochondrial surveys undertaken in Europe (Arca et al. 2015; Budge et al. 2017; Granato et al. 2019; Jones et al. 2020; Takahashi et al. 2019), and with the hypothesis of a single mated-queen propagule introduced into Europe (Arca et al. 2015). The nuclear data further supports the assertion of a French source, as microsatellite alleles identified in the peninsular populations are a subset of the alleles found in France, which in turn are a subset of the alleles found in Asia (Arca et al. 2015). A similar finding has been reported by the other single microsatellite surveys conducted up to now outside of France (Budge et al. 2017; Jones et al. 2020). Furthermore, the F_{ST} values indicate that the peninsular populations are more closely related to the French population than to any other Asian population. This is in agreement with the assignment test, which assigned every single individual to the French population with a 100% score and a high probability.

Observational data suggest that *V. velutina* crossed the French borders, into Spain and Italy, at the northwestern edges of the Pyrenes and the Alps (Bertolino et al. 2016; Goldarazena et al. 2015; López et al. 2011). The STRUCTURE analysis performed herein supports these dispersal pathways, with the individuals from Italy and the Basque Country (Spain) clustering with the French population. In contrast, the founding populations early detected in Portugal, far from the invading front in the Basque Country (Goldarazena et al. 2015; López et al. 2011), could have originated from independent propagules, consistent with mounting evidence that multiple introductions from native sources are common in biological invasions (reviewed in Bossdorf et al. 2005; Dlugosch and Parker 2008; Roman and Darling 2007; Wares et al. 2005). However, our data does not support a scenario of multiple introductions pointing instead to a long-distance jump into Portugal, as has been documented for the United Kingdom (Budge et al. 2017; Jones et al. 2020). Whether the propagule originated from the core population in France or from the leading edge, at the time located in the Spanish Basque Country, cannot be determined by this dataset. However, there are rumours that the invading propagule hitchhiked with a pine wood cargo imported from the Landes region (France) into Viana do Castelo (Portugal) in 2010 (M. Maia, pers. comm.). This mechanism of long-distance jump has greatly accelerated the invasion of Europe, earlier by expanding the southwesternmost edge in Portugal (Grosso-Silva and Maia 2012) and more recently the northwesternmost edge in the United Kingdom (Budge et al. 2017; Jones et al. 2020).

Descendants of the Portuguese founding population rapidly established and spread throughout the Atlantic coast southwards and northwards into the neighbouring Spanish province of Galiza (Carvalho et al. 2020). The first sightings of *V. velutina* in Galiza, only one year after its official arrival in Portugal, occurred in A Mariña, over 200 km north from the founding nucleus in Viana do Castelo and 400 km west from the invading front in the Spanish Basque Country (Goldarazena et al. 2015; López et al. 2011). Considering an average dispersal rate of $\sim$ 40 km year^{-1} in the Iberian Atlantic coast (Carvalho et al. 2020), the introduction in A Mariña was most likely assisted by humans. Interestingly, STRUCTURE places the individuals sampled in the region of A Mariña in the Portuguese blue cluster, contrasting with all neighbouring individuals which belong to the French orange cluster. The genetic pattern shown in Fig. 4 suggests that Galiza is a place of secondary contact between two fronts: one stemming from the founding population in Portugal and the other from the natural range expansion along the Atlantic coast in northern Iberia (see the dynamic map in http://frelonasiatique.mnhn.fr/). Whether the individuals sampled in the region of A Mariña with ancestry in the blue cluster derive from the founding propagule detected in Galiza in 2012 or represent recent human-mediated migration with origin in the Portuguese population is uncertain and deserves closer scrutiny.

Founder or range expansion events are usually accompanied by losses of genetic diversity, which can be more or less severe depending on propagule size (Nei et al. 1975) or on the influx of migrants from the core into edge populations (Swaegers et al. 2013). The impact of the founder event on neutral diversity was of intermediate intensity in the French population of *V. velutina*, as it retaining 85.2% of the allelic richness and 79.9% of the heterozygosity present in the putative source population of China (Arca et al. 2015). This level of diversity loss is in the range documented for numerous animal and plant invasions, with varying propagule pressures, and was claimed to be linked to their invasion success (reviewed in Bossdorf et al. 2005; Dlugosch and Parker 2008;

Roman and Darling 2007; Wares et al. 2005). Empirical support for this claim comes from a meta-analysis on plants and animals that found a significant positive effect of genetic diversity on measures of colonization (Forsman 2014).

Although founder diversity is not the only factor determining the fate of introduced propagules, it is a critical one as it will influence the adaptive response of fitness-related traits to the novel environment (Drake and Lodge 2006; Lockwood et al. 2005). Remarkably, owing to the polyandrous mating system in *V. velutina*, the single multi-mated queen that gave rise to the invasion in Europe was able to bring in an important fraction of neutral, and perhaps fitness-related, variation (Arca et al. 2015). This trait, together with other extraordinary life history traits common to social Hymenoptera (reviewed in Beggs et al. 2011; Moller 1996), probably helped establishment and spread of her descendants in Europe, similar to invasions of the little fire ant *Wasmannia auropunctata*, the eastern honey bee *Apis cerana*, or the bumble bee *Bombus terrestris*, which also started from a few introduced queens (Ding et al. 2017; Eloff et al. 2020; Mikheyev et al. 2009; Schmid-Hempel et al. 2007).

More severe was the bottleneck associated to the secondary founder event in Portugal, as reflected by the significant losses of 29.6% and 53.9% in allelic richness and heterozygosity, respectively. Notably, when the values were computed relative to the French source, diversity loss was more pronounced in this secondary founder event (Ar = 17.4%; uHe = 42.3%) than in the original introduction in France (Ar = 14.8%; uHe = 20.1%). Nevertheless, the founding propagule was still able to establish and spread throughout the Atlantic coast of Iberia, although at a slower pace than in France (Carvalho et al. 2020). Given suitable climatic conditions and prey abundance in the area (Carvalho et al. 2020; Villemant et al. 2011), it is possible that this diversity impoverishment might have contributed to deceleration of the invasion.

While microsatellite variation is not necessarily a proxy for fitness-related diversity (Reed and Frankham 2001), it is possible that the sex locus suffered further depletion during the secondary founding event, hampering population growth. Similar to other haplodiploid hymenopteran species, in *V. velutina*, sex is determined by a multiallelic locus in which females develop from heterozygous genotypes whereas males from hemizygous (Heimpel and De Boer 2008). When allelic diversity is low at the sex locus, there is a higher chance that the queen will produce homozygous eggs, which will develop into diploid males. The problem is that these males represent a heavy burden to the colony because, in addition to usually being sterile, they are produced at the expense of female workers but do not contribute to colony tasks. Studies of nests collected in the United Kingdom and France documented a high frequency of diploid males, which is consistent with low diversity at the sex locus and inbreeding among the members of the small founding population (Budge et al. 2017; Darrouzet et al. 2015; Jones et al. 2020).

While the invasion in Portugal arose from a secondary founder event, the greater levels of genetic diversity in Spain and Italy, as well as the clustering patterns (Figs. 3 and 4), suggest a process of natural expansion from the core population in France. Leading-edge populations usually lose diversity at lower rates than founding populations because they are buffered against the effects of genetic drift by gene flow from the core population, especially in cases of organisms with high dispersal rate as for *V. velutina* (Shi and Chen 2012; Swaegers et al. 2013). Contrary to expectations from ecological niche modelling, the geographical extent of the invasion in Italy is very modest as compared to that of Iberia (Bertolino et al. 2016; Carvalho et al. 2020; Rome et al. 2011; Villemant et al. 2011). While several interacting biotic and abiotic factors (e.g., clime, prey abundance, interspecific competition, and control measures) may explain differential invasion dynamics between the two peninsulas, genetic factors associated to the secondary introduction in Portugal have likely played an important role in population growth in Iberia. In less than 6 years, the primary front expanded throughout the north Atlantic coast in Spain all the way to Galiza where it met the secondary front originating from Portugal (http://frelonasiatique.mnhn.fr/). Detection of six first-generation migrants in Portugal suggests that the primary front is expanding southward and this movement is bringing in new genetic variation, as reflected by the 20.33% increase in allelic richness (Fig. 5).

Regardless of the many details that remain elusive, our genetic analysis suggests that *V. velutina* invaded north-western Spain and Italy by a process of natural range expansion whereas the invasive process in Portugal stemmed from a long-distance jump. While severe losses of genetic variability from serial

bottlenecks did not avert the successful spread of *V. velutina* in Portugal, the recent secondary contact between the two invading fronts in Galiza is helping restoring diversity levels, which may increase population fitness and boost invasiveness. However, the findings presented herein come from selectively neutral markers and whether these can be used as surrogates for adaptively important variation is a controversial matter (Reed and Frankham 2001). A recent sharp rise in the number of detected nests in Portugal denotes population growth (Carvalho et al. 2020). Whether this observation is due to improved fitness related with the influx of genetic variation carried by the primary expanding wave or to mechanisms involving phenotypic plasticity, epigenetic modifications, de novo mutations, purging of deleterious mutations, and/or increase of additive variance at ecologically important traits remains to be known (see the review of Estoup et al. 2016).

In this study, we unveiled the patterns of neutral diversity in populations of the Italian and Iberian peninsulas, thereby expanding current knowledge on the invasion genetics of *V. velutina*. However, a more complete understanding of the molecular mechanisms underpinning the phenomenal invasion of this hornet in Europe requires that functionally important diversity is scrutinized and this can be done using high-throughput sequencing. Unfortunately, the whole genome of *V. velutina* has not yet been sequenced and therefore the genomic resources for this type of study are still lacking.

Conclusion

This study builds upon the seminal analysis of the founding propagule in France (Arca et al. 2015) to greatly expand current understanding on the population genetics aspects underlying the phenomenal invasion of *V. velutina* in Europe. Invading events in southern Europe have been achieved by leading edge expansion and long-distance jump. Remarkably, the genetic impoverishment exhibited by the invading populations, which was more severe in the secondary founding population in Portugal than in Spain and Italy, did not preclude successful population establishment and spread, encouraging further research into the mechanisms underlying invasiveness in social Hymenoptera. Equally remarkable is the substantial gain in genetic diversity introduced by a low number of first-generation migrants, suggesting that the signature of genetic drift can be rapidly erased by gene flow and this can occur rapidly in species like *V. velutina*, with high dispersal abilities. Our findings further stress the importance of surveillance and control measures to halt gene flow, and call for more stringent policies within the European Union preventing circulation of propagules, which may lead to secondary founder events favouring establishment and expansion of alien organisms.

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Author contributions MAP conceived the ideas and designed the methodology. AQ performed the laboratorial work as well as most of the analyses with assistance of DH and MAP. All the authors contributed with data interpretation. MAP and AQ wrote the manuscript. DH, JG, XM and LB critically revised the manuscript for important intellectual content.

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Availability of data and material All the data and materials are available in the manuscript and in the supplementary tables.

Code availability (software application or custom code) Not applicable.

Declarations

Conflict of interest The authors have no conflict of interest to declare that are relevant to the content of this article.

References

Arca M, Capdevielle-Dulac C, Villemant C, Mougel F, Arnold G, Silvain J-F (2012) Development of microsatellite markers for the yellow-legged Asian hornet, *Vespa velutina*, a major threat for European bees. Conserv Genet Resour 4:283–286

Arca M et al (2015) Reconstructing the invasion and the demographic history of the yellow-legged hornet, *Vespa velutina*, in Europe. Biol Invasions 17:2357–2371

Barbet-Massin M, Rome Q, Muller F, Perrard A, Villemant C, Jiguet F (2013) Climate change increases the risk of invasion by the Yellow-legged hornet. Biol Conserv 157:4–10. https://doi.org/10.1016/j.biocon.2012.09.015

Barbet-Massin M, Rome Q, Villemant C, Courchamp F (2018) Can species distribution models really predict the expansion of invasive species? PLoS One 13:e0193085. https://doi.org/10.1371/journal.pone.0193085

Barbet-Massin M, Salles JM, Courchamp F (2020) The economic cost of control of the invasive yellow-legged Asian hornet. NeoBiota 55:11–25. https://doi.org/10.3897/neobiota.55.38550

Beggs JR, Brockerhoff EG, Corley JC, Kenis M, Masciocchi M, Muller F, Rome Q, Villemant C (2011) Ecological effects and management of invasive alien Vespidae. Biocontrol 56:505–526

Bertolino S, Lioy S, Laurino D, Manino A, Porporato M (2016) Spread of the invasive yellow-legged hornet *Vespa velutina* (Hymenoptera: Vespidae) in Italy. Appl Entomol Zool 51:589–597. https://doi.org/10.1007/s13355-016-0435-2

Bessa AS, Carvalho J, Gomes A, Santarém F (2016) Climate and land-use drivers of invasion: predicting the expansion of *Vespa velutina nigrithorax* into the Iberian Peninsula. Insect Conserv Divers 9:27–37. https://doi.org/10.1111/icad.12140

Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1–11. https://doi.org/10.1007/s00442-005-0070-z

Budge GE et al (2017) The invasion, provenance and diversity of *Vespa velutina* Lepeletier (Hymenoptera: Vespidae) in Great Britain. PLoS One 12:e0185172. https://doi.org/10.1371/journal.pone.0185172

Carvalho J, Hipólito D, Santarém F, Martins R, Gomes A, Carmo P, Rodrigues R, Grosso-Silva J, Fonseca C (2020) Patterns of *Vespa velutina* invasion in Portugal using crowdsourced data. Insect Conserv Divers 13:501–507. https://doi.org/10.1111/icad.12418

Daly D, Archer ME, Watts PC, Speed MP, Hughes MR, Barker FS, Jones J, Odgaard K, Kemp SJ (2002) Polymorphic microsatellite loci for eusocial wasps (Hymenoptera: Vespidae). Mol Ecol Notes 2:273–275. https://doi.org/10.1046/j.1471-8286.2002.00220.x-i2

Darrouzet E, Gévar J, Guignard Q, Aron S (2015) Production of early diploid males by European colonies of the invasive hornet *Vespa velutina nigrithorax*. PLoS One 10:e0136680. https://doi.org/10.1371/journal.pone.0136680

Demichelis S, Manino A, Minuto G, Mariotti M, Porporato M (2014) Social wasp trapping in north west Italy: Comparison of different bait-traps and first detection of *Vespa velutina*. Bull Insectology 67: 307–317. ISSN 1721-8861

Ding G, Xu H, Oldroyd B, Gloag R (2017) Extreme polyandry aids the establishment of invasive populations of a social insect. Heredity 119:381–387. https://doi.org/10.1038/hdy.2017.49

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Drake JM, Lodge DM (2006) Allee effects, propagule pressure and the probability of establishment: risk analysis for biological invasions. Biol Invasions 8:365–375. https://doi.org/10.1007/s10530-004-8122-6

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree argania spinosa skeels endemic to Morocco. Theoret Appl Genetics 92:832–839. https://doi.org/10.1007/BF00221895

Elder R, Bell K (1998) Establishment of *Chilocorus spp*. (Coleoptera: Coccinellidae) in a *Carica papaya* L. orchard infested by *Aonidiella orientalis* (Newstead) (Hemiptera: Diaspididae). Aust J Entomol 37:362–365. https://doi.org/10.1111/j.1440-6055.1998.tb01597.x

Eloff J, Veldtman R, Bulgarella M, Lester P (2020) Population genetics of the invasive wasp *Vespula germanica* in South Africa. Insectes Soc 67:229–238. https://doi.org/10.1007/s00040-020-00752-x

Estoup A, Ravigné V, Hufbauer R, Vitalis R, Gautier M, Facon B (2016) Is there a genetic paradox of biological invasion? Annu Rev Ecol Evol Syst 47:51–72. https://doi.org/10.1146/annurev-ecolsys-121415-032116

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform. https://doi.org/10.1177/117693430500100003

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Forsman A (2014) Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. PNAS 111:302–307. https://doi.org/10.1073/pnas.1317745111

Goldarazena A, De Heredia IP, Romon P, Iturrondobeitia JC, Gonzalez M, Lopez S (2015) Spread of the yellow-legged hornet *Vespa velutina nigrithorax* du Buysson (Hymenoptera: Vespidae) across Northern Spain. EPPO Bulletin 45:133–138. https://doi.org/10.1111/epp.12185

Goudet J (2005) Hierfstat, a package for R to compute and test hierarchical F-statistics. Mol Ecol Notes 5:184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x

Granato A, Negrisolo E, Bonomi J, Zulian L, Cappa F, Bortolotti L, Mutinelli F (2019) Recent confirmation of a single haplotype in the Italian population of *Vespa velutina*. Biol Invasions 21:2811–2817. https://doi.org/10.1007/s10530-019-02051-4

Grosso-Silva JM, Maia M (2012) *Vespa velutina* Lepeletier, 1836 (Hymenoptera, Vespidae), new species for Portugal. Arquiv Entomol 6:53–54

Hasegawa E, Takahashi J (2002) Microsatellite loci for genetic research in the hornet *Vespa mandarinia* and related species. Mol Ecol Notes 2:306–308. https://doi.org/10.1046/j.1471-8286.2002.00224.x

Haxaire J, Tamisier JP, Bouguet JP (2006) Vespa velutina Lepeletier 1836, une redoutable nouveauté pour la faune de France (Hym., Vespidae). Bull Soc Entomol Fr 111:194

Heimpel GE, De Boer JG (2008) Sex determination in the Hymenoptera. Annu Rev Entomol 53:209–230. https://doi.org/10.1146/annurev.ento.53.103106.093441

Husemann M, Sterr A, Maack S, Abraham R (2020) The northernmost record of the Asian hornet *Vespa velutina nigrithorax* (Hymenoptera, Vespidae). Evol Syst 4:1. https://doi.org/10.3897/evolsyst.4.47358

Jones EP, Conyers C, Tomkies V, Semmence N, Fouracre D, Wakefield M, Stainton K (2020) Managing incursions of *Vespa velutina nigrithorax* in the UK: an emerging threat to apiculture. Sci Rep 10:1–8. https://doi.org/10.1038/s41598-020-76690-2

Keeling MJ, Franklin DN, Datta S, Brown MA, Budge GE (2017) Predicting the spread of the Asian hornet (*Vespa velutina*) following its incursion into Great Britain. Sci Rep 7:1–7. https://doi.org/10.1038/s41598-017-06212-0

Kenis M, Auger-Rozenberg M-A, Roques A, Timms L, Péré C, Cock MJW, Settele J, Augustin S, Lopez-Vaamonde C (2009) Ecological effects of invasive alien insects. Biol Invasions 11:21–45. https://doi.org/10.1007/s10530-008-9318-y

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour 15:1179–1191. https://doi.org/10.1111/1755-0998.12387

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Laurino D, Lioy S, Carisio L, Manino A, Porporato M (2020) *Vespa velutina*: an alien driver of honey bee colony losses. Diversity 12:5. https://doi.org/10.3390/d12010005

Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. Trends Ecol Evol 20:223–228. https://doi.org/10.1016/j.tree.2005.02.004

López S, González M, Goldarazena A (2011) *Vespa velutina* lepeletier, 1836 (Hymenoptera: Vespidae): first records in Iberian Peninsula. EPPO Bull 41:439–441. https://doi.org/10.1111/j.1365-2338.2011.02513.x

Martín-Hernández R et al (2018) *Nosema ceranae* in *Apis mellifera*: a 12 years postdetection perspective. Environ Microbiol 20:1302–1329. https://doi.org/10.1111/1462-2920.14103

Mikheyev A, Bresson S, Conant P (2009) Single-queen introductions characterize regional and local invasions by the facultatively clonal little fire ant *Wasmannia auropunctata*. Mol Ecol 18:2937–2944. https://doi.org/10.1111/j.1365-294X.2009.04213.x

Moller H (1996) Lessons for invasion theory from social insects. Biol Conserv 78:125–142. https://doi.org/10.1016/0006-3207(96)00022-5

Monceau K, Arca M, Leprêtre L, Mougel F, Bonnard O, Silvain J-F, Maher N, Arnold G, Thiery D (2013a) Native prey and invasive predator patterns of foraging activity: the case of the yellow-legged hornet predation at European honeybee hives. PLoS One 8:e66492. https://doi.org/10.1371/journal.pone.0066492

Monceau K, Maher N, Bonnard O, Thiéry D (2013b) Predation pressure dynamics study of the recently introduced honeybee killer *Vespa velutina*: learning from the enemy. Apidologie 44:209–221. https://doi.org/10.1007/s13592-012-0172-7

Monceau K, Bonnard O, Thiéry D (2014) *Vespa velutina*: a new invasive predator of honeybees in Europe. J Pest Sci 87:1–16. https://doi.org/10.1007/s10340-013-0537-3

Monceau K, Maher N, Bonnard O, Thiéry D (2015) Evaluation of competition between a native and an invasive hornet species: Do seasonal phenologies overlap? Bull Entomol Res 105:462–469. https://doi.org/10.1017/S0007485315000280

Mutinelli F et al (2014) Detection of *Aethina tumida* Murray (Coleoptera: Nitidulidae.) in Italy: outbreaks and early reaction measures. J Apic Res 53:569–575. https://doi.org/10.3896/IBRA.1.53.5.13

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. Evolution 29:1–10. https://doi.org/10.2307/2407137110

Neumann P, Carreck NL (2010) Honey bee colony losses. J Apic Res 49:1–6. https://doi.org/10.3896/IBRA.1.49.1.01

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Mol Ecol 13:55–65. https://doi.org/10.1046/j.1365-294X.2004.02008.x

Paine T, Millar J (2002) Insect pests of eucalypts in California: implications of managing invasive species. Bull Entomol Res 92:147–151. https://doi.org/10.1046/j.1365-294X.2004.02008.x

Peakall R, Smouse P (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and researchdan update. Bioinformatics 28:2537–2539

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. Heredity 95:536–539. https://doi.org/10.1093/jhered/esh074

Poidatz J, Monceau K, Bonnard O, Thiéry D (2018) Activity rhythm and action range of workers of the invasive hornet predator of honeybees *Vespa velutina*, measured by radio frequency identification tags. Ecol Evol 8:7588–7598. https://doi.org/10.1002/ece3.4182

Porporato M, Manino A, Laurino D, Demichelis S (2014) *Vespa velutina* Lepeletier (Hymenoptera Vespidae): a first assessment two years after its arrival in Italy. Redia 97:189–194

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data.

Genetics 155:945–959. https://doi.org/10.1093/genetics/155.2.945
Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. PNAS 94:9197–9201. https://doi.org/10.1073/pnas.94.17.9197
Reed DH, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095–1103. https://doi.org/10.1111/j.0014-3820.2001.tb00629.x
Requier F, Garnery L, Kohl PL, Njovu HK, Pirk CW, Crewe RM, Steffan-Dewenter I (2019) The conservation of native honey bees is crucial. Trends Ecol Evol 34:789–798. https://doi.org/10.1016/j.tree.2019.04.008
Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225
Robinet C, Suppo C, Darrouzet E (2017) Rapid spread of the invasive yellow-legged hornet in France: the role of human-mediated dispersal and the effects of control measures. J Appl Ecol 54:205–215. https://doi.org/10.1111/1365-2664.12724
Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454–464. https://doi.org/10.1016/j.tree.2007.07.002
Rome Q, Perrard A, Muller F, Villemant C (2011) Monitoring and control modalities of a honeybee predator, the yellow-legged hornet *Vespa velutina nigrithorax* (Hymenoptera: Vespidae). Newslett IUCN/SSC Invasive Species Special Group Aliens 31:7–15
Rome Q, Dambrine L, Onate C, Muller F, Villemant C, García-Pérez A, Maia M, Carvalho-Esteves P, Bruneau E (2013) Spread of the invasive hornet *Vespa velutina* Lepeletier, 1836, in Europe in 2012 (Hym., Vespidae). Bull Soc Entomol Fr 118:21–22
RStudio Team (2015) RStudio: integrated environment development for R, Boston, Massachusetts http://www.rstudio.com/
Schmid-Hempel P, Schmid-Hempel R, Brunner P, Seeman O, Allen G (2007) Invasion success of the bumblebee, *Bombus terrestris*, despite a drastic genetic bottleneck. Heredity 99:414–422. https://doi.org/10.1038/sj.hdy.6801017
Shi M-M, Chen X-Y (2012) Leading-edge populations do not show low genetic diversity or high differentiation in a wind-pollinated tree. Popul Ecol 54:591–600. https://doi.org/10.1007/s10144-012-0332-7
Smit J, Noordijk J, Zeegers T (2018) Will the Asian hornet (*Vespa velutina*) settle in the Nederlands? Entomolog Ber 78:2–6
Steinhauer N, Kulhanek K, Antúnez K, Human H, Chantawannakul P, Chauzat M-P (2018) Drivers of colony losses. Curr Opin Insect Sci 26:142–148. https://doi.org/10.1016/j.cois.2018.02.004
Swaegers J, Mergeay J, Therry L, Larmuseau M, Bonte D, Stoks R (2013) Rapid range expansion increases genetic differentiation while causing limited reduction in genetic diversity in a damselfly. Heredity 111:422–429. https://doi.org/10.1038/hdy.2013.64
Takahashi J, Okuyama H, Kiyoshi T, Takeuchi T, Martin SJ (2019) Origins of *Vespa velutina* hornets that recently invaded Iki Island. Jpn Jersey Island, UK Mitochondrial DNA A DNA Mapp Seq Anal 30:434–439. https://doi.org/10.1080/24701394.2018.1538366
Turchi L, Derijard B (2018) Options for the biological and physical control of *Vespa velutina nigrithorax* (Hym.: Vespidae) in Europe: a review. J Appl Entomol 142:553–562. https://doi.org/10.1111/jen.12515
Valière N (2002) GIMLET: a computer program for analysing genetic individual identification data. Mol Ecol Notes 2:377–379. https://doi.org/10.1046/j.1471-8286.2002.00228.x-i2
Van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x
Villemant C, Haxaire J, Streito JC (2006) Premier bilan de l'invasion de *Vespa velutina* Lepeletier en France (Hymenoptera, Vespidae). Bull Soc Entomol Fr 111:535–538
Villemant C, Barbet-Massin M, Perrard A, Muller F, Gargominy O, Jiguet F, Rome Q (2011) Predicting the invasion risk by the alien bee-hawking Yellow-legged hornet *Vespa velutina nigrithorax* across Europe and other continents with niche models. Biol Conserv 144:2142–2150. https://doi.org/10.1016/j.biocon.2011.04.009
Wares JP, Hughes AR, Grosberg RK (2005) Mechanisms that drive evolutionary change: Insights from species introduction and invasions. In: Species invasions : insights into ecology, evolution, and biogeography. Sinauer Associates Sunderland, MA, pp 229–257
Witt R (2015) Erstfund eines Nestes der Asiatischen Hornisse *Vespa velutina* Lepeletier, 1838 in Deutschland und Details zum Nestbau (Hymenoptera, Vespinae). Ampulex 7:42–53