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Reconstructing the invasion and the demographic history of the yellow-legged hornet, *Vespa velutina*, in Europe

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Abstract The yellow-legged hornet, *Vespa velutina*, was accidentally introduced from Southeast Asia and then invaded France and Korea over the last 10 years. Since its introduction, its predation on honeybee colonies has rapidly become an economic problem in invaded countries. Using mitochondrial cytochrome C oxidase and 22 nuclear microsatellite loci, we showed that native hornet populations were well differentiated and highly diverse. In contrast, introduced populations from France and Korea suffered a genetic bottleneck, which did not prevent their rapid geographic expansion. Analysis of the genetic data indicates that French and Korean populations likely arose from two independent introduction events. The most probable source population is from an area between the Chinese provinces of Zhejiang and Jiangsu. This invasion route is in agreement with knowledge on trade and historical records. By studying colonies of *V. velutina*, we demonstrated its polyandry, which is very rare among Vespidae. This mating behavior could have favored the success of this Asian hornet in Europe and Korea. Combined, the population and colony results suggest that very few or possibly only one single multi-mated female gave rise to the invasion.

Keywords Yellow-legged hornet · *Vespa velutina* · Invasive species · Approximate Bayesian computation

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Introduction

The increases of global trade and human mobility over the last century have resulted in unprecedented numbers of invasions by non-native species all over the world. These biological invasions have severe impacts on agriculture and natural resources (Mack et al. 2000). Social insects are among the most successful invasive groups (Moller 1996). Their invasive success has been attributed to life-history traits that facilitate their introduction and expansion into new habitats (Holway et al. 2002). Sociality is generally associated with behavioral flexibility, which may contribute to the invasive success of some social species. For example, introductions of ants, which are among the most widespread and damaging alien species, are often accompanied by alterations in social behavior and colony structure that contribute to their success (Tsutsui et al. 2001; Suarez and Tsutsui 2008).

Another key to the invasive success of social insects is the ability of a single queen to found a large colony and to produce many reproductive females that then offer a large dispersal capacity (Moller 1996; Chapman and Bourke 2001; Schmid-Hempel et al. 2007; Mikheyev et al. 2009).

Association with human activities such as pollinisation is another element that makes social insects successful invaders because of deliberate displacement of large samples of individuals and often repetitive events of introduction. This association is particularly relevant for bees (*Bombus* and *Apis* spp.), which have been introduced to many new areas for economic reasons (Moller 1996; Ings et al. 2006).

Among social hymenoptera, *Vespa* species are not commonly considered invasive. Several species have been introduced to areas beyond their native ranges,

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but most failed to become established (Beggs et al. 2011; Villemant et al. 2011). One Vespidae that has made rapid and high-profile invasions is the yellow-legged hornet, *Vespa velutina*, recently introduced and established in both France and Korea. This was the first successful invasion of an exotic vespid into Europe (Villemant et al. 2011) and it was noticeable for its velocity. *Vespa velutina* naturally occurs in Asia, from Afghanistan to eastern China, Indo-China and Indonesia (Carpenter and Kojima 1997), where it is known as an active predator of honeybees (Abrol 1994; Tan et al. 2007). The first field record of the yellow-legged hornet in France was obtained in the Lot-et-Garonne *département* in 2005, although, according to local testimonies, it was likely present as early as 2004 (Villemant et al. 2011). Following its introduction, the hornet spread rapidly across southwest France. The invaded area had reached about 190,000 km² by 2010 (Villemant et al. 2011; Fig. 1) and 360,000 by 2012 (Rome et al. 2013). The hornet was also reported in northern regions of Spain and in northern Portugal. One male was also recorded in Belgium in 2011 (Rome et al. 2013). Comparisons of the climatic niches between the native and invasive range revealed that many countries of Western Europe have climatic conditions suitable for *V. velutina* and, therefore, a high probability of being invaded (Villemant et al. 2011). The first hornet observation in France was made by a French bonsai producer who regularly imported pots from Chinese coastal areas close to Shanghai (Villemant et al. 2011). It has thus been suggested that the first introduction of the Asian yellow-legged hornet into France was associated with an importation of horticultural pots carried on cargo boats from these Chinese provinces (Villemant et al. 2006). It is well known that social Vespidae are transported passively by man, as fertilized queens seek sheltered locations in which to undergo diapause. Such shelters are often found in human goods, which may then be transported to new locations with the queens as passengers (Beggs et al. 2011). A full nest could not voyage in this way as, under the same conditions, it would be detected and destroyed.

Little is known about the *V. velutina* introduction to Korea. The hornet was found for the first time on the Bongrae Mountain, Yeong-do districts, near Busan, in 2003. *Vespa velutina* appears to have spread much more slowly in Korea (Choi et al. 2012) than in France
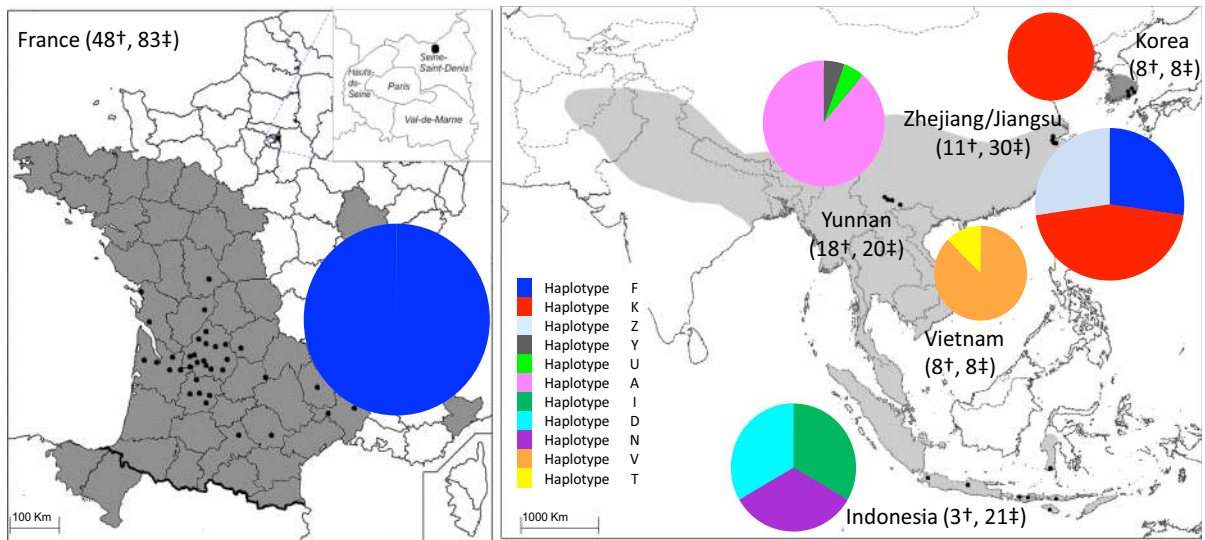
Fig. 1 Map of the six regions sampled for the yellow legged hornet *Vespa velutina*. *Each point* on the map represents a sampled locality. *Grey areas* indicate the known distribution range of *V. velutina* in 2010. The *pie charts* display haplotype frequency for the mitochondrial COI marker in each sampled region and their size is proportional to the sampling effort. The haplotype names are associated with a color code as indicated in the legend. *Dagger* number of samples used for mitochondrial analysis; *double dagger* number of samples for microsatellite analysis. All samples are listed in table S1 in which samples used for mtDNA characterization are in *bold*

(Rome et al. 2013). The reason for this might be that the invasive hornet must contend with six other *Vespa* species in Korea, whereas only one, *V. crabro*, is present in France (Villemant et al. 2011; Choi et al. 2012). *Vespa velutina* is currently abundant in the southern part of Korea and has spread over one-third of the country as a whole (Choi et al. 2013).

Like many other invasive species, *V. velutina* has never caused significant economic problems in its native range, where it is likely that many biotic factors (e.g., predation and competition) control its population levels. To our knowledge, such factors are absent from the area of France where this species was introduced. Multiplication of the species has also probably been greatly enhanced by the abundance of the European honeybee *Apis mellifera* throughout the whole of Europe (Perrard et al. 2009; Monceau et al. 2013). *Apis mellifera* is probably the hornet's main prey in Europe because, in contrast with the Asian species *Apis cerana* (Villemant 2008; Ken et al. 2005), this honeybee has no effective defensive behavior to fight against its new predator (Rortais et al. 2010; Monceau et al. 2013). Yellow-legged hornets feed on a diversity of insects and may thus also have a significant impact on other native arthropods (Villemant et al. 2011). The impact of *V. velutina* on insect diversity is difficult to assess, while the damage to French apiaries is obvious: high losses due to deaths of colonies and to reduction of honey production has led some beekeepers to cease apiculture activities (Villemant et al. 2011). In addition to damages caused to apiaries, *V. velutina* is alarming to human populations due to its huge nest and population size.

The objective of this study is to genetically trace the history of the introduction of *V. velutina* in France and Korea in order to (1) identify the geographic origin of the introduced propagules, (2) estimate the number of founders responsible for the establishment of the invasive population, and (3) improve knowledge about the life history traits of *V. velutina* that may have facilitated its demographic success in the invaded areas.

Materials and methods

Sample collection and DNA isolation

For the population study, a total of 170 samples of *V. velutina* were obtained from six geographic areas

(Fig. 1): France (FRA, n = 83), Korea (KOR, n = 8) (invaded areas), Vietnam (VIE, n = 8), Indonesia (IND, n = 21), Yunnan (YUN, n = 20) and Zhejiang/Jiangsu provinces (ZHE/JIA, n = 30) (native areas) (Table S1). Females were collected in front of hornet nests, in front of beehives or using beekeeper's traps, containing a food attractant. When a sweeping net was used in front of the nests, only one female per nest was analyzed. For the hornets captured away from their nests (in beekeeper's traps or in front of beehives), only one sample per locality was genotyped from localities situated at least 3 km away from one another in order to limit the chance of sampling individuals from the same colony. Because a restricted number of individuals were sampled from each region, individuals were treated as representative samples from each country studied. Consequently, the term 'population' refers to the total number of individuals from each sampled country (or province in the case of China). Mean distance between samples was 99 km in France, 53 km in Korea, 3 km in Vietnam, 591 km in Indonesia, 177 km in Yunnan and 60 km in Zhejiang/Jiangsu.

In addition to the isolated hornets collected in the field for population genetic studies, we analyzed samples of V. velutina from 10 different colonies in order to determine the number of progenitors (reproductive females and their mates). Colony samples were collected in three areas of France: Dordogne, Gironde and Ile-de-France. A total of 396 females were DNA-genotyped. DNA was extracted from pupae or adults (thorax or legs) using the 'DNeasy tissue Kit' (Qiagen).

Mitochondrial DNA sequencing and analysis

Mt DNA of 48 individuals among the 83 V. velutina from France and 48 among the 87 samples from Asia (Indonesia, Vietnam, China and Korea) was amplified using universal primer sequences HCO-2198 and LCO-1490 to yield a 658 bp fragment of the mitochondrial gene cytochrome C oxidase subunit I (COI) (Folmer et al. 1994). Samples were chosen to cover the widest geographic range as possible (Table S1).

Polymerase Chain Reaction (PCR) amplifications were performed according to the standard PCR reaction protocol used at the Canadian Centre for DNA Barcoding (Hajibabaei et al. 2005). PCR products were then verified on a 2 % agarose gel. Purified PCR

fragments obtained from the HCO/LCO primer pair were sequenced in both directions. Both strands of DNA were aligned manually using BIOEDIT 7.0.5.3 (Hall 1999). Edited sequences of each haplotype were deposited in GenBank (accession numbers: JQ780449 JQ780462). Haplotype and nucleotide diversity (Nei 1987) were calculated using DnaSP 4.10.9 (Rozas et al. 2003). A haplotype parsimony network was reconstructed using TCS 1.21 (Clement et al. 2000), as described by Templeton et al. (1992), with a probability cut-off set at 95 %.

Microsatellite amplification and analysis

Seven microsatellite loci previously developed for other Vespidae were used for V. velutina (Hasegawa and Takahashi 2002, Daly et al. 2002). Fifteen additional loci isolated from V. velutina were used in the present study (Arca et al. 2011). All 170 hornets were genotyped at 22 microsatellite loci (listed in Table S1, Supplementary materials).

PCR amplifications were performed as described in Arca et al. 2011. PCR products were analyzed in an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems). Alleles were scored with GeneMapper v.4.1 (Applied Biosystems), and each allele-call was checked manually. Due to amplification problems with heterologous loci (List2003, VMA8, VMA6, LIST2018B and LIST2004B), we could not obtain complete genotypes for 30 of the Chinese, the 8 Korean and 15 of the Indonesian specimens (Table S1). As full genotype was obtained for the remaining samples, all 22 loci were used for following analyses.

Number of alleles and allele frequencies were calculated using GenAlEx 6.5 (Peakall and Smouse 2012) and exact tests of Hardy Weinberg equilibrium were performed using Genepop (Rousset 2008).

Population structure

Population structure was analyzed using Bayesian clustering techniques in STRUCTURE v.2.0 (Pritchard et al. 2000). The STRUCTURE analysis was performed according to Falush et al. 2003, considering both the admixture model and the correlated allele frequencies between populations. Following recommendations by Gilbert et al. (2012), the length of the burn-in and MCMC (Monte Carlo Markov chain) were both 100,000. The number of clusters (K) was set from

1 to 10. For the whole dataset (170 hornets distributed between six populations), 20 runs were carried out for each value of K. Runs were performed twice: first, including genotypes from the 22 markers and, second, keeping genotypes from only the 15 markers developed specifically for *V. velutina*. Results from 20 runs were merged with CLUMPP (Jakobsson and Rosenberg 2007) and visualized using custom R scripts.

Genetic differentiation between populations was estimated using F_{st} calculated in ARLEQUIN v. 3.01 (Excoffier et al. 2005) and tested with 10 000 random permutations of genotypes.

Bottleneck detection

We investigated the occurrence of recent genetic bottlenecks in the French population using a test of heterozygosity excess implemented in Bottleneck 1.2.0.2 (Cornuet and Luikart 1996; Piry et al. 1999). Heterozygosity excess is expected in populations that have experienced a significant reduction in size because rare alleles are lost (Cornuet and Luikart 1996). Two mutation models were tested: the infinite allele model (IAM) and the two-phase model (TPM), the latter of which incorporates elements of the IAM and stepwise mutation model (SMM) (variance = 12, SMM = 95 %, Piry et al. 1999). The Wilcoxon sign-rank test was used to test for a statistically significant bottleneck (Luikart and Cornuet 1998).

Assignation test

The program GeneClass v.2.0 (Piry et al. 2004) was used to assign or exclude reference populations (Indonesia, Vietnam, Yunnan and Zhejiang/Jiangsu) as possible origins of individuals from France and Korea on the basis of multilocus genotypes; we used the standard criterion described by Rannala and Mountain (1997). The Monte Carlo resampling method (Paetkau et al. 2004) was also applied to identify the accurate critical values of exclusion/inclusion: our results were based on 10 000 simulated genotypes for each population and a threshold probability value of 0.01.

Mating statistics

For this study, 12 microsatellite loci were used (shown in bold and underlined in Table S2, Supporting information). Queen and mate genotypes were inferred from the worker offspring data using Colony 2.0.1.1 (Wang and Santure 2009). When available, males were also genotyped and known queen alleles were indicated in Colony 2.0.1.1 (the haploid genotypes of males are the direct product of queen meiosis). Four trials were performed with varying assumed genotyping error rates (0.001, 0.01, 0.05 and 0.1). Allele frequencies inferred from the population study were used as input.

Inferring native range and invasion scenarios, estimating the number of foundresses and introduction date using approximate Bayesian computation

In addition to traditional population genetic approach we used the DIYABC program v.1.0.4.46 (Cornuet et al. 2008) to explore putative scenarios of invasion followed by the yellow-legged hornet in France and Korea using Approximate Bayesian Computation (ABC) method (Appendix 1 4, supplementary material). This approach was also used to estimate the effective population size of the French invasive population and the specific contribution of males and females. According to the biology of the species, founder diploid females mate with haploid males before wintering. Furthermore, in this study, mating statistics indicated that polyandry is frequent in *V. velutin*a, at least in France. Finally, mitochondrial and nuclear data and multiple mating behavior suggest that, if the invasion was due to females mated before introduction, there should be mixing between one or a few female genomes but many more male genomes. This should leave a signature in the allele frequency distributions that appears bimodal. The mode at low allelic frequencies results from the less bottlenecked male founders population that partly maintained rare alleles, while the mode at high frequencies results from the more bottlenecked female population which retained only major alleles. Since the DIYABC program is able to detect admixture from frequency distribution statistics, it should detect this feature as an admixture signature between one bottlenecked population (the females) and one outbred population (the males). Accordingly, we chose to refine an invasion scenario to estimate the specific contribution of males and females. In the chosen scenario, the source of the invasive French population was a

population close to ZHE/JIA, this population sharing more alleles with the French sample than any other native population sampled. In the refined scenario, the French invasive population (Pinv) was founded by admixture between two sources artificially modeled as independent populations, corresponding to the founder females (Pfm) and their male mates (Pm), with different sizes (*Nfm* and *Nm*, respectively). As the male contribution was intimately associated with the female one (spermatozoids are carried by females in their spermatheca), admixture was expected to occur immediately at the foundation of the invasive population with following rates: 2/3 for diploid females and 1/3 for haploid males, corresponding to the expected contribution of each genomic source at equilibrium in the invasive population. In the supplementary file 1 is given the R code calculating the female contribution at each generation. In this scenario, only ZHE/JIA and the invasive population from France were considered. Prior settings for parameters and summary statistics were the same as for scenario comparison (see Appendix 3 and 4, Supporting information). The model was named the "sexual admixture model".

Results

Among native populations, only eight individuals could be obtained from Vietnam (VIE). This sample was however maintained in the analyses to increase the coverage of hornet native range. Some bias in allelic diversity may have arisen from this small sample size, so results from the VIE population are therefore only mentioned and not thoroughly discussed.

Mitochondrial DNA analysis

Mitochondrial haplotypes were characterized for four native regions (Zhejiang/Jiangsu, Yunnan, Vietnam and Indonesia) and two invaded regions (Korea and France), as well as for the closely related species *Vespa bicolor*, which was used as an outgroup. The alignment yielded a 658 bp fragment with 120 (18.24 %) variable sites, among which 42 were parsimony-informative. Eleven COI haplotypes were detected from a total of 96 *V. velutina* individuals sampled in native and invaded regions (Fig. 1; Fig. S1, Supporting information). All 11 haplotypes were

found in the native area but only two of them in the introduced areas (Fig. 1), making haplotype diversity significantly higher in the native range. Furthermore, each native population showed at least two haplotypes, with haplotype diversity ranging from 0.216 to 1 and nucleotide gene diversity ranging from 0.001 to 0.038, while both indices were 0 in the invaded areas. All sequences in France conformed to a single haplotype F and all those in Korea to another single haplotype, K (Fig. 1).

Both haplotypes F and K were also found in the native population of Zhejiang/Jiangsu but not in the other sampled regions (Yunnan, Vietnam and Indonesia), suggesting that the invasive populations are closer to this native population than to the other sampled native populations.

Microsatellite diversity

All 22 microsatellite loci analyzed in this study were polymorphic in at least one population. In France, only one locus was monomorphic (D2-142) (Tables S2 and S3, Supporting information).

No linkage disequilibrium was observed for any pair of loci after correction for multiple testing. The subsequent analyses were therefore performed on multi-locus data from all twenty-two microsatellites. Two regions (Korea and eastern China) showed no departure from Hardy Weinberg equilibrium (HWE) at any of their loci. In the four other populations, significant deviations were observed at atleast one locus. However, the departure was not systematically observed over all the loci in one population or over all the populations at one locus (Table S3). Two hypotheses may be invoked to explain these departures: the presence of population-specific null alleles and the occurrence of substructuration within populations. The latter explanation is likely, as departure from HWE was observed in populations sampled over large distances that may be subject to Walhund effect (France, Yunnan and especially Indonesia).

The mean number of alleles detected per locus per population varied from 2.7 in Korea to 7.3 in Yunnan (Table S3). Mean number of alleles per locus and expected heterozygosity were significantly higher in the native range than in introduced regions (*P* value of Wilcoxon's sign rank test comparing France or Korea on one side and Zhejiang/Jiangsu, Yunnan or Indonesia on the other were below 0.05 for both indices after

correction for multiple testing, Benjamini and Yeku-tieli 2001).

Bottleneck detection

The decrease in genetic diversity observed in the invasive populations may be indicative of a bottleneck event. The heterozygosity excess indicating a genetic bottleneck was tested only on the French population, the sample size of Korea being too small. The result was consistent with a bottleneck: heterozygosity excess was significant ($P < 0.05$) under both IAM and TPM models.

Furthermore we visualized mode-shifts in the distribution of allele frequencies using the graphical method described in Luikart et al. (1998). Populations with stable size are characterized by an L-shaped distribution, indicative of many alleles with low frequencies. Bottlenecked populations are supposed to show a mode-shift to more alleles at intermediate frequencies, resulting from the elimination of rare

alleles by founder effect. The allelic distribution in French and Korean populations revealed skewed distributions with a bimodal tendency and a "wave" at high allele frequencies (Fig. 2) indicative of bottleneck events according to Luikart et al. (1998) method.

Population structure and relationships

There was considerable divergence among populations: pairwise F_{st} ranged from 0.078 to 0.365 and all values were significantly different from zero (Table 1). Generally, F_{st} values were high between native and invasive populations (except between Korea and Zhejiang/Jiangsu) and always slightly higher between French and native populations than between Korea and native populations. For both invasive populations, the lowest F_{st} estimate was found with the Zhejiang/Jiangsu population. The F_{st} value between French and Korean invasive populations (0.33) was larger than the values between each of them and the Zhejiang/Jiangsu population (0.21 and

Fig. 2 Histograms of allele frequencies over all microsatellite loci for each of the invasive (FRA, KOR) and native (IND, VIE, YUN, ZHE/JIA) populations studied. Sample size (Na) is the total number of alleles. *Bottlenecks* are expected to shift the mode from low to high frequencies (i.e. from left to right)
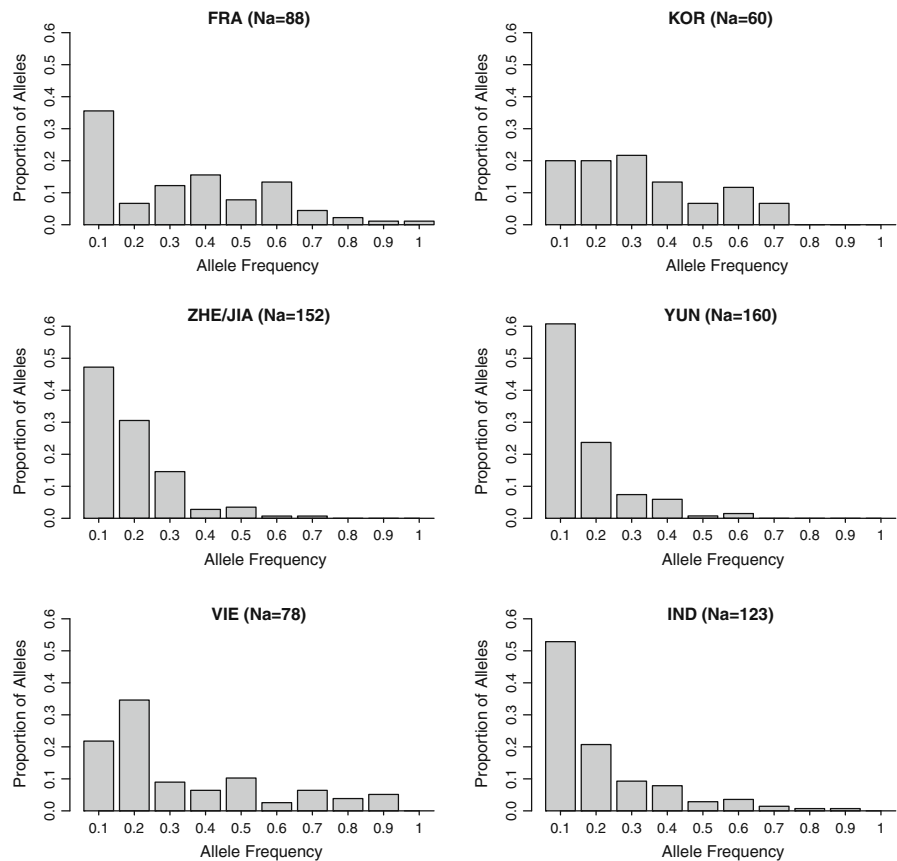
Table 1 Pairwise genetic differentiation for two introduced and four native populations of *Vespa velutina*

	FRA	KOR	ZHE/JIA	YUN	VIE	IND
FRA	0		0.7368	0.0112	0.0000	0.0005
KOR	0.3298*	0	0.5702	0.0027	0.0000	0.0000
ZHE	0.2087*	0.0887*	0			
YUN	0.2274*	0.1620*	0.0784*	0		
VIE	0.3649*	0.3233*	0.2121*	0.1937*	0	
IND	0.3244*	0.2385*	0.1534*	0.1481*	0.3053*	0

F_{st} are indicated below the diagonal. Statistically significant values are indicated with an asterisk. The mean log assignment likelihood (LogL) of individuals from two invasive populations (France and Korea) to the four native populations are indicated above the diagonal (calculated by GeneClass 2.0. Piry et al. 2004). Invasive populations are shown in bold type

0.09 for France and Korea, respectively), suggesting two independent introductions from the native area into France and Korea.

Progressive partitioning using STRUCTURE is visualized in Fig. 3, with presentation of successive subdivisions obtained when increasing K from 2 to 10. Despite the long run length and high number of repeats, no clustering pattern converged, as shown by the multimodal solution for some individuals, mainly from Yunnan and Zhejiang/Jiangsu. Nevertheless, some clusters coherent with geographical origin emerged when K was increased. The French invasive population was the first to separate from the native populations. The Indonesian population also broke away very early (from K = 3) and in a stable manner, despite the extent of the sampling area. Vietnam separated clearly from the other populations at K = 9. Geographical structure was not clearly resolved in Chinese populations. However, the two Chinese populations seem separated and some sub-clusters are likely to be found in Yunnan. The Korean invasive population clustered with native populations and specifically with the sample of Zhejiang/Jiangsu until K = 9. Above this value, Korean individuals clustered apart. Similar clustering emerged when the analysis was performed using only the 15 markers developed from *V. velutina* (Figure S2, Supplementary materials).

Invasive *V. velutina* sampled in France and Korea were mostly assigned by GeneClass to the eastern Chinese province of Zhejiang/Jiangsu (Table 1). Individuals from these two invasive populations were rarely if ever assigned to the populations from Indonesia, Vietnam and Yunnan.

Genetic composition of colonies

Each of the sampled colonies had worker offspring from a single queen (Table 2). Depending on the assumed genotyping error rate, the total estimated number of patrilines varied from 22 (error rate = 0.05) to 37 (error rate = 0.001) over the 9 colonies studied. Even with the highly permissive 10 % error rate, 7 out of the 9 queens appeared polyandrous. Furthermore, the true error rate was most probably low due to double analysis of suspect genotypes (for example unique genotypes within a queen progeny).

The mean number of matings observed per queen was between 2.44 and 4.11 and patrilines may have reached 8 in nest V0926. This result demonstrated that multiple mating is the rule in *V. velutina* at least in France (Table 3).

Number of founders and introduction date

The number of introduced foundresses, their number of mates and the date of the first introduction were inferred under the sexual admixture model using the DIYABC program. Considering the high genetic proximity found between Zhejiang/Jiangsu population and France (also supported by DIYABC study, Appendix 5, Figure S3 and Table S4 and S5, Supplementary material), this native population was considered as the invasion source. Both the average relative bias and the RMSE were low and did not indicate any systematic over- or underestimation of the various parameters. None of the estimated parameters, except *Ninv*, had bias or RMSE higher than
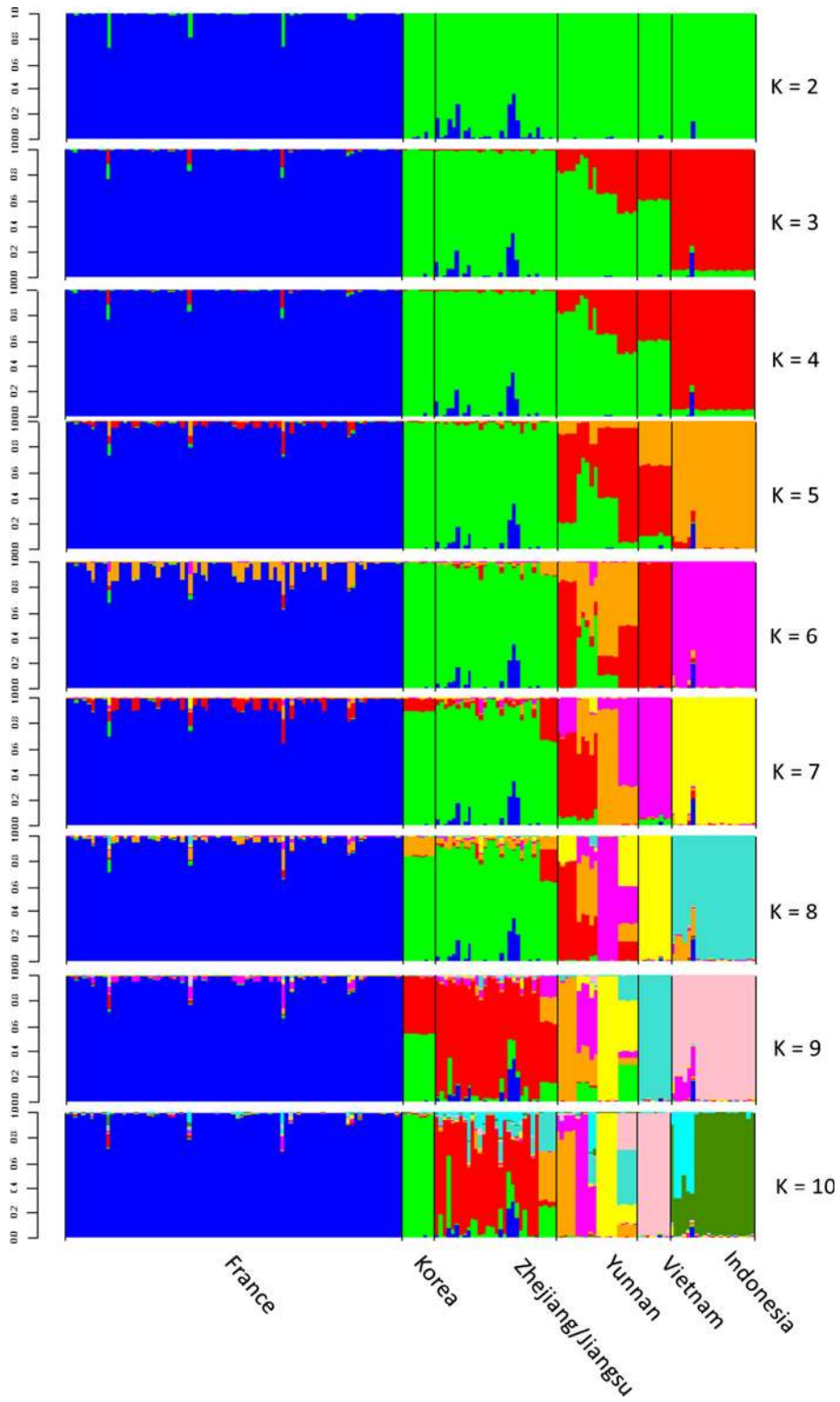
Fig. 3 Graphical output from STRUCTURE (Pritchard et al. 2000) for each value of K from 1 to 10 [modified in DISTRUCT (Rosenberg et al. 2002)]. Each *vertical line* represents an individual, and the *color* composition displays the probability of belonging to each of the 2 10 clusters defined by STRUCTURE

Table 2 Number of queens and mates per colony in the French population, inferred from the worker offspring data using Colony 2.0.1.1

	M n	F 2n	NLoc	Nq	Nm
V0809	0	51	10	1	1 3
V0813	10	48	8	1	3 5
V0825b	25	42	11	1	1 3
V0830	0	41	12	1	3 6
V0907	20	43	12	1	2 5
V0912	0	13	8	1	2 3
V0921	0	45	8	1	1 2
V0926	5	76	11	1	5 8
V0927	0	36	12	1	2 4
All nests					2.44 4.11

M n number of analyzed males per colony, *F 2n* number of analyzed females per colony, *NLoc* number of analyzed loci per individual, *Nq.* number of queens per colony, *Nm* number of mates per queen (range variation is given between the four analyses with different genotyping error rates)

one. In general, the width of the 50 % credible intervals was small, showing that the parameters were reasonably well estimated; all values of the parameter estimates resulting from the pseudo-observed data sets lie between 50 and 200 % of the estimated median values. The lack of confidence in the estimate of present effective size of the French population may have resulted from the expanding status of this population, as the hornet is still colonizing new territories in France and Europe. Nevertheless, confidence was far larger for other parameters. From this sexual admixture model, we estimated the number of introduced females as only 1 (0.99) and its number of mates as 1.8 diploid males, corresponding to 3.6 haploid males. The time of introduction into France (*ti*) was estimated at 7.48 generations, corresponding to between 7 and 8 years before sampling.

Discussion

The primary objective of this study was to genetically characterize the introduction events of *Vespa velutina* in Europe and Korea in order to infer the number of introduced populations, their origins and their sizes. Results are more reliable for France, as sampling effort was larger for this invaded territory than for Korea, though general conclusions may apply to both invasion histories. A recent study that used larger sample sizes from Korean populations confirms these general conclusions (Choi et al. 2013).

Geographical origin of invaders

Several studies have shown that identifying a source population precisely can be challenging if the potential source region is large, genetically homogeneous or not differentiated (Wares et al. 2005). In our study, genetic differentiation between the native populations was high providing theoretically optimal conditions for accurate source determination (Guillemaud et al. 2011). When differentiation between populations is high, a large sampling effort may be required to ensure that the real source population is not missed. We only obtained sparse samples from the native range of the species and the precise geographic origin of invasive population has to be confirmed. Nonetheless, our results strongly suggest that the origin of the Korean and the French invasive populations of *V. velutina* originated from an eastern Chinese population. The two mitochondrial haplotypes detected in each invaded area to date were also found in two neighboring localities (37 km apart) in the two Chinese provinces of Jiangsu and Zhejiang. Furthermore, analysis of nuclear markers indicates that the recently established French and Korean populations are more closely related to the eastern Chinese population than to the other Asian populations. These findings were also in agreement with the assignment test conducted with GeneClass. The high percentage of individuals from the invaded areas assigned to the Zhejiang/Jiangsu population and the high significance of these assignments suggest that the more likely source for introduction among the available samples is this eastern Chinese coastal area. Approximate Bayesian Computation also suggested a population source closely related to Zhejiang/Jiangsu. The inferred eastern China origin of the French invasive population analyzed here is consistent with factual information about the commercial exchanges that were probably responsible for the introduction of *V. velutina* into France. Such an event is also in agreement with the increased importance of Asia as a source of alien hymenopteran species in Europe (Rasplus et al. 2010). Nevertheless we have to take into account the fact that we only obtained sparse samples from the native range of the species. A larger sampling effort may be

Table 3 Prior settings, parameter estimations (median of the posterior distribution) and confidence in these estimations for the scenario devoted to inferring the number of introduced individuals and the date of the first introduction in France

Parameter	Priors	Estimates		Bias	RMSE	50 % cov	95 % cov	Fact 2
Ns	U [1 10000]	[median	3.2×10^3]	0.217	0.894	0.532	0.962	0.878
$t1$	U [1 10]	[median	7.48]	0.236	0.976	0.554	0.954	0.854
$Ninv$	U [1 10000]	[median	9.38×10^2]	**2.57**	**18.237**	0.512	0.948	0.716
Nm	U [1 20]	[median	1.80]	0.021	0.318	0.538	0.962	0.988
Nfm	U [1 20]	[median	9.90×10^{-1}]	0.084	0.38	0.506	0.938	0.986

The validity of the parameter estimates was evaluated with DIYABC by comparing the estimates obtained from the ABC analysis of 100 pseudo observed datasets (pods) simulated from parameter values drawn from the prior distributions and scenario 1 (Appendix 1 4, Table S5 supplementary material) with the true value of the parameters (i.e. the values drawn from the priors). Then the relative mean square error (RMSE: the square root of the average square difference between the 100 ABC estimates and the actual parameter value) and the relative bias (the mean absolute difference between the 100 ABC estimates and the actual parameter value divided by the actual parameter value), the 90 and the 50 % coverage (i.e. the fraction of 90 and 50 % highest posterior densities obtained from the 100 ABC analyses that includes the actual parameter value), and the factor 2 (the proportion of the 100 ABC estimates obtained that are within the range of X/2 and 2X, with X the actual parameter value)

Ns Zhejiang/Jiangsu effective population size. $t1$ time of introduction to France. $Ninv$ French effective population size. Nm effective number of haploid males that mated with introduced founders. Nfm effective number of introduced foundresses. Note that the estimate of Nfm is outside the prior distribution range. This an artifact due to the linear regression used in the ABC procedure. The estimate is therefore considered to be one in the following. Strongly biased and/or imprecise estimates are highlighted in bold. U [X,Y] refers to as a uniform distribution with X and Y as lower and upper limits, respectively

required to ensure that the real source population has not been missed.

Estimated number of introduced individuals

A notable characteristic of the invasive hornet populations in France and Korea is their impoverished genetic diversity compared with native populations from Asia. A single mtDNA haplotype was found in each of these invasive populations, while 2 3 haplotypes were sampled in populations from the native range. Genetic diversity at microsatellite loci was also lower in all invasive populations than in native populations, with less than half the allelic richness, and lower levels of heterozygosity. Furthermore, native populations were well differentiated from each other, with even some substructuration observed in the Yunnan area. In contrast, the French population appeared as a single homogeneous genetic group for any number of clusters tested. Choi et al. (2013) studied larger samples from two localities in Korea (44 and 43 samples, respectively) with 7 of the microsatellite markers included in the present study. Their results are in complete agreement with the results we obtained: (1) they showed a clear depletion of genetic diversity in Korea compared with native populations,

(2) they found evidence of a genetic bottleneck when testing with BOTTLENECK software, and (3) they did not detect any genetic structure in the Korean population.

Taken together, highly reduced levels of genetic variation in introduced *V. velutina* populations along with significant bottlenecks and the lack of related population structure suggest that this hornet experienced a single and severe founder event in both Korea and France.

Several aspects of the data suggested that the strength of the bottleneck in Europe arose from the introduction of very few or possibly a single foundress. This hypothesis is supported by the presence of a single mitochondrial haplotype in France. Indeed, each colony is generally founded by a single diploid female, resulting in the presence of a unique mtDNA in the colony and future queens. Results associated with nuclear markers are, however, not compatible with the introduction of a singly-mated female for a number of reasons. First, more than three alleles are observed at many loci in France. Second, the drop-off in diversity is more pronounced in mitochondrial DNA than in nuclear DNA contrarily to what is described in other species that have experienced a similar invasion pattern (Mikheyev

et al. 2009). Combined mitochondrial and nuclear data suggest that the establishment of the present population of yellow-legged hornet in France would have been made possible by the introduction of very few or, as suggested by the DIYABC analysis, a single queen fertilized by several males. The discovery of frequent polyandry in *Vespa velutina* favors this scenario.

We used an ABC approach to test the hypothesis that the invasion of France originated from the introduction of a small number of founder females fertilized by several males. The ABC analyses confirmed that all of the French hornet samples were likely derived from a single female, and the effective number of their haploid male mates (Pm) was estimated at 3.6 (Cl = [2.00 17.26]). This result is consistent with the independent 'Colony' analysis from which we estimated that females in France are fertilized by 2.44 4.11 males. A precise estimation could not be obtained for the invasive Korean population, due to small sample size.

Genetic bottlenecks and loss of substantial amount of genetic diversity are common features of invasive populations (Puillandre et al. 2008; Dlugosch and Parker 2008). Another famous case of successful invasion with a large decrease in diversity in the introduced range concerns *Bombus terrestris*, the European bumblebee. In Tasmania, for example, an invasion was apparently caused by the introduction of a very small number of individuals from a single source region, perhaps as few as two bees (Schmid-Hempel et al. 2007). The high success of some species, despite such large genetic losses is a well-known paradox (Dlugosch and Parker 2008).

Invasive success despite a small propagule size

Several studies on conservation genetics have demonstrated that reduced genetic variation due to genetic drift following a founder effect limits the ability of a population to adapt, and small population size increases the risk of extinction (Frankham and Ralls 1998; Allendorf and Lundquist 2003). Particularly in isolated haplodiploid populations, where sex is often determined by a single locus, increased homozygosity due to inbreeding increases the production of non-viable or effectively sterile diploid males, which reduces population growth rates and effective sizes, thus potentially creating a rapid extinction vortex (Zayed et al. 2007; Dlugosch and Parker 2008). The

invasion of the yellow-legged hornet in France is an exemplary case of invasive success from a very small propagule size. Paradoxically, *V. velutina* appears to be able to establish even after a severe genetic bottleneck resulting from the introduction of very few or possibly a single female (although multiply mated). Furthermore, the species has succeeded in two apparently independent cases of introduction: in Europe and in Korea. Several species of ant and wasp have become highly invasive in their introduced range, but have generally not experienced reductions in propagule pressure on the same scale as *V. velutina* (Tsutsui and Suarez 2003). A rare example is the case of the little fire ant *Wasmannia auropunctata*, for which there is evidence that only a single female or a small group of sisters was introduced to Central Africa and Hawaii. The unusual, largely clonal, reproductive strategy of *W. auropunctata* may have enhanced its success as an invasive species (Mikheyev et al. 2009).

Admittedly, many biological and environmental factors might have contributed to the invasiveness of *V. velutina* in France and counterbalanced the negative effect on genetic diversity of small number of female foundresses; these might include the suitable climatic conditions, the abundance of honeybees and, according to Villemant et al. (2011), the low level of competition from other *Vespa* sp. that *V. velutina* faces in Europe. Additionally, our results also point to the contribution that reproductive system traits of this species may have made to the success of the invasion, by increasing the number of male founders.

In this paper, we demonstrated that *V. velutina* is a polyandrous species. According to a recent data compilation (Hughes et al. 2008), *V. velutina* is the only *Vespa* species to date to present moderate polyandry (defined by the authors as 2 10 effective mates), whereas *V. crabro* and *V. mandarina* exhibit facultative low polyandry and *V. ducalis* is monoandrous. Available data highlight the fact that *V. velutina* queens exhibit one of the highest mean effective mating frequencies among the Vespidae. Interestingly, among the few other Vespidae species showing moderate polyandry, *Vespula germanica* and *Vespula vulgaris* also revealed to be invasive species (Beggs et al. 2011). Similarly, the successful invasion of leaf-cutting ant *Acromyrmex octospinosus* in Guadeloupe is likely the result of a multimated female (Mikheyev 2008). Despite the cost of multiple mating, polyandry could be beneficial to colonies, not

only because monogynous queens need sperm from several males in order to produce enough workers, but also because it may offer several other genetic advantages that may have determined the success of invasion (Crozier and Fjerdingstad 2001). Notably, multiple mating implies higher genetic variation among offspring (Yasui 1998), which can improve the resistance of the whole colony to parasites or pathogens (Schmid-Hempel and Crozier 1999) and increase colony survival under variable environmental conditions (Jennions and Petrie 2000); this may be of importance for adaptation to a new environment in an invaded area. Genetic diversity reduction observed in a population developed from a single female is far less severe when this female is multiply mated than when it is only singly mated. As a result, polyandry may also decrease diploid male load at the colony level by decreasing the production of diploid males resulting from the sex determination mechanism (Jennions and Petrie 2000).

Conclusion

The results of this study, based on a number of highly informative genetic markers, provide the first genetic reconstruction of the *V. velutina* invasion in Europe. The congruence between the results based on the different classes of markers and different analytical approaches adds weight to the credibility of our estimates.

The species has clearly proven its high invasive potential: our genetic study shows that the introduction of a very small number or even probably a single multi-mated queen can initiate a full-scale invasion. This discovery represents a challenge to management practices intended to control such introductions or to remove them from non-native environments. Rapid detection at the port of entry seems to be the best way to prevent a large scale infestation, although just one escaped founder, or a very small number, can render management actions ineffective.

It is possible that other invasive species share these characteristics and could become invaders even if only a very small number of individuals are introduced. It is therefore a priority to distinguish these species so that they can be managed with particular caution, as traditional practices may be insufficient for their control.

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