Journal of Insect Physiology 109 (2018) 93–99

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Comparison of reproductive traits of foundresses in a native and an invasive hornet in Europe

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ARTICLE INFO

Keywords:
Vespa velutina
Vespa crabro
Spermatheca
Sperm stock
Ovarian maturation
Fat reserves
Species competition

ABSTRACT

The population dynamics of annual social hymenoptera such as vespids depend largely on the fertility of the foundresses, which, in turn, is a key factor in the context of biological invasions. The native European hornet *Vespa crabro* (*Vc*) and the invasive Asian hornet *Vespa velutina* (*Vv*) have generally similar ecological traits, e.g. nesting and feeding habits, although they differ in colony size, which is higher in *Vv*. Furthermore, in contrast to *Vc*, *Vv* is more specialized in its predatory habits, intensively hunting honey bees at the hive. Comparing the morphological and reproductive traits of two closely related species occupying the same ecological niche, one of which is a native species and the other an alien, can help us to gain an understanding of the invasion process. To this end, we here compare reproductive (ovarian size and maturation, fat level, spermatheca size and sperm stock, fecundity) and morphological traits (head size, weight) of the foundresses of these two hornet species. We observed that ovarian maturation began approximately one month earlier in *Vv* than in *Vc*, and that the fat level in the former was lower. We found twice the number of sperm in the mated foundresses of *Vv* than in those of *Vc* (more than 100×10^3 and less than 50×10^3 sperm, respectively), in a 16% smaller spermatheca in *Vc*. Furthermore, the sperm of *Vv* was found to be 65% shorter than that of *Vc*. The precocity and higher potential fecundity of *Vv* queens may have favoured this species over *Vc* in terms of predatory behaviour, and thereby contributed to its invasiveness.

1. Introduction

The proliferation of an alien insect generally has detrimental effects on native species in the invaded area, particularly in terms of competitive exclusion when they have similar ecological niches (Mooney and Cleland, 2001). Reproductive potential is recognized as key factor in understanding population dynamics and the potential for invasiveness (see, Moller, 1996; Sakai et al., 2001 for a review). We might expect alien species to possess promoting traits linked to invasiveness (such as dispersal, fecundity etc) more developed than non-invasive ones, that will enhance population growth, including dispersal, establishment, and proliferation (Hudina et al., 2014; Chapple et al., 2012; Holway and Suarez, 1999; Blackburn et al., 2009; Weis, 2010; Monceau et al., 2015a). These life traits allow invasive species to outcompete the local species, thereby facilitating more successful invasion. Given the adaptability conferred by their sociality, social insects are good candidates for biological invasions (Moller, 1996; Suarez et al., 1999; Cervo et al., 2000; Beggs et al., 2011). The European hornet *Vespa crabro* (Lin. 1758) is the only hornet originally distributed in Continental Europe (Archer, 1994). This species is protected in some European countries for its ecological value (for example in Germany), and was considered an endangered species even before the arrival of *Vespa velutina* in Europe (Erlandson, 1988). The Yellow-legged Asian hornet, *Vespa velutina* var. *nigrithorax* (Lepelletier 1835), is native to East Asia, and was accidentally introduced to South France from China around 2004 (Monceau et al., 2014; Arca et al., 2015). The introduced *V. velutina* (*Vv*) subsequently spread to neighbouring European countries, including Spain, Italy, Portugal, Belgium, and Germany, and has more recently been recorded in England and Scotland (Monceau and Thiéry, 2016). The ecological niche of *Vv* is very similar to that of *V. crabro* (*Vc*): they both hunt arthropods, are scavengers, and consume ripe fruits. However, due to its larger colonies and outbreaks in the invaded areas, *Vv* has an enhanced impact on the local biodiversity. Furthermore, although reports of predation on domestic honeybees by *Vc* are largely anecdotal (Baracchi et al., 2010), *Vv* predates them in huge amounts, including both imagoes and larvae (Matsuura and Yamane, 1990; Monceau et al.,

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https://doi.org/10.1016/j.jinsphys.2018.07.004
Received 23 October 2017; Received in revised form 22 June 2018; Accepted 9 July 2018
Available online 10 July 2018
0022-1910/

2013; Monceau et al., 2018; Tan et al., 2007). The mass hunting of bees by *Vv* is therefore an additional threat to the beekeeping industry, which is already in crisis owing to multifactorial causes (Oldroyd, 2007; Flynn, 2008; Brown, 2009; Kluser et al., 2010; Le Conte et al., 2010). Several species of Asian hornets are known to attack honeybee colonies, using collective hunting strategies of varying degrees of sophistication (Matsuura et al., 1990), even if hives do represent a risky resource to exploit, since honeybees are defensive and can sometime kill the hunters (Tan et al., 2012; Arca et al., 2014). One hypothesis for the paucity of bees predation by *Vc* is that a *Vc* nest produces low numbers of workers, which is not conducive to risky attacks on hives. The capacity to produce large number of workers could thus be a key factor in optimal exploitation of such a resource. Monceau et al. (2015a) showed that the seasonal phenologies in *Vv* and *Vc* overlaps to some extent. Even if they do not compete directly for food sources, either direct or indirect interspecific competition between these two species at the initiation stage is likely. *Vc* prefers cryptic sites for nest construction (Langowska et al., 2010), whereas mature *Vv* nests are mostly found in open sites, typically in tree canopies (Monceau et al., 2013, 2014). Nevertheless, numerous *Vv* colonies are initiated in roofs and underground, and two months later, some of those colonies relocate to more open sites (Matsuura and Yamane, 1990). During this critical period of nest initiation, it seems probable that interspecific competition would occur. Monceau et al. (2015b) compared several behaviours of *Vc* and *Vv* foundresses, including aggressiveness and exploration, and showed that *Vv* outperforms *Vc* in such traits, which can be advantageous for invasion and competition with *Vc*. In the present study, we compared different fertility traits of the two hornet species that are prerequisites for larger colonies, and could thus increase or reduce the impact of the invasive *Vv,* not only on *Vc,* but also on honeybees.

The fertility of social Hymenoptera with an annual cycle, such as *Vespa* species, depends on different criteria, notably the number of eggs produced (Fletcher and Ross, 1985; Reeve, 1991; Reeve and Nonacs, 1992; Foster et al., 2004), physiological investment in reproduction through ovarian development (Cini et al., 2013; Makino, 2016), and precocity in establishing and developing a colony (Monceau et al., 2015a). In *Vespa* species, the eggs are laid by a single queen, whereas hormonal castration of the workers prevents them from mating and laying eggs (Foster et al., 2000). If the queen disappears for any reason, some workers can undergo ovarian maturation but will lay only unfertilized eggs (Matzura and Yamane, 1990). All females develop from fertilized eggs (diploid), whereas the males develop from unfertilized eggs (haploid). The queen's fertility therefore influences the size and the structure of the colony (Takahashi et al., 2002), and sperm stored by queens after copulation are an essential resource for the production of workers and colony growth (Elbassiouny, 2007; Page and Metcalf, 1982). In this regard, sperm morphology should also be taken into consideration because longer sperm occupies more space, and thus an equal spermathecal volume would contain less long sperm than short sperm.

Hornet gynes remain in their nest for a short period after emergence to increase their fat reserves, which will serve for both hibernation and egg production (Matsuura, 1984; Matsuura and Yamane, 1990; Martin, 1993). After mating, the gynes conserve the sperm compacted in their spermatheca for the rest of their lives. The size and shape of the female spermatheca are species-specific (Gotoh et al., 2008), and in Vespidae comprises a single epithelial layer, subdivided into three main regions: a globular reservoir, a spermathecal duct, and a Y-shaped spermathecal gland (Martins et al., 2005). After hibernating, and a short period of food collection, ovarian maturation occurs for the duration of reproductive life (Matsuura, 1984; Matsuura and Yamane, 1990; Makino, 2016). The queen (foundress) subsequently establishes a colony, and gradually uses spermatozoa to fertilize (or not) the eggs she is laying.

The objective of this study was to answer the following questions related to the fertility of queens of two European hornet species, the native *V. crabro* and the invasive *V. velutina*. Obtaining such data helps understanding of the invasiveness of *Vv* but is also of general interest, as it addresses the problem of biological invasions. (i) What is the proportion of foundresses that are fertilized in spring in each species? (ii) Are there differences between *V. velutina* and *V. crabro* in the spermatheca and the characteristics of its contents? (iii) Are there differences in the timing of ovarian maturation in the two species? Moreover, to date, the reproductive biology of hornets in Europe has been poorly investigated. This study is based on an examination of 237 hornet queens (184 *Vv* and 53 *Vc*) in which we measured size and weight, described their sexual maturation, measured their spermatheca diameter, and assessed the amount and morphology of spermatozoa contained in their spermathecae.

2. Material and methods

2.1. Insects

Foundresses of the species *Vespa velutina* (VV) and *V. crabro* (VC) were collected from 9th March 2015 to 21st May 2015 (VV = 182, VC = 22), and from 18th March 2016 to 11th May 2016 (VV = 107, VC = 31). All the foundresses were captured using bottle food traps (lager containing 5% red fruit syrup) at different locations in the vicinity of Bordeaux (France) (for more details, see Supplementary data, Table 1). The traps were surveyed two or three time a week, and only alive foundresses were used in this study. Traps were located under the shade of trees to prevent queens to die by heat, and topped with a plastic roof to prevent the trap content from rainfalls. Once captured, foundresses were placed individually in meshed boxes using a 30 cm long clamp. Before being dissected, the insects were maintained for 1 to 48 h in plastic boxes (10 × 20 × 15 cm) within a climatic chamber at 23 ± 1 °C, LD 12/12, and were provided with *ad libitum* water and honey.

2.2. Dissection and measurements

The hornets were killed by cooling in a freezer for 5 min, so that they could be weighed using an electronic balance (AS 220/C/2; Radwag 2011, Poland) immediately prior to dissection. Five min was a maximum duration to prevent a potential sperm degradation by ice crystallization.

2.2.1. Head width

The head width of the hornets was measured using an electronic calliper (0–150 mm, Stainless hardened, e = 0.01 mm) at the largest distance between the eyes.

2.2.2. Fat level

Under a stereomicroscope (OLYMPUS SZ61), the abdomen of the insect was separated from the remainder of the body, and was then immobilized with dorsal face uppermost on a dissection surface. The sternites were then removed, thereby enabling the fat level to be assessed, as described for *Polistes* in Beani et al. (2011). The amount of fat was classified based on a scale from 0 to 4, with 0 indicating the absence of extra fat and 4 representing an abdomen filled with fat. All the measurements were performed by a single experimenter (JP) to limit observer bias.

2.2.3. Ovary development

After removing the superficial abdominal fat with clamps, the number of ovarioles that constitute each ovary was assessed. Ovarian development was assessed by evaluating the stage of the ovarioles, as described in Beani et al. (2011). This evaluation is illustrated in Fig. 1. To assess if the queen had already laid eggs, we looked for the remains of yellow bodies in the lower portion of the flat ovarioles (S), as evidence of previous egg laying. The number of eggs in each ovariole ready to be laid was also counted. To determine if an egg was ready (O), we

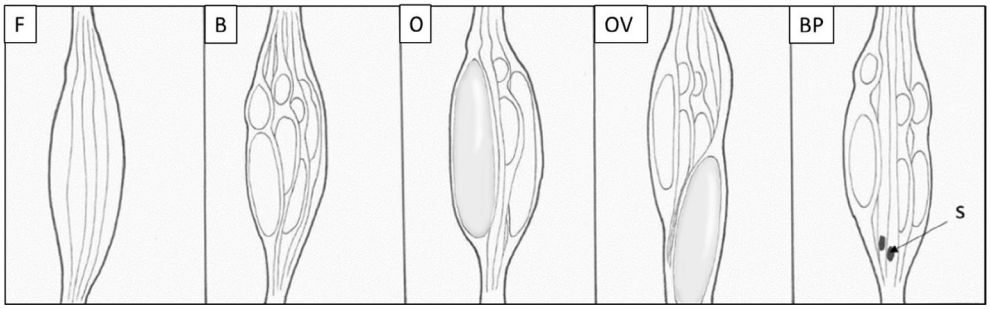

Fig. 1. Ovariole development stages: F: flat ovarioles (one side), B: oocytes in formation, O: eggs, OV: eggs already in the oviduct, BP: no egg ready, but egg(s) already laid, S: spots of yellow body residuals from previously laid eggs (Schema J. Poidatz).

initially examined its colour: eggs at this stage change from a cream colour to a characteristic pearly colour. We also compared the size of the egg with the average size of the eggs that could already be observed in the tractus (stage OV).

We pooled the catches by week after first catch each year for this analysis, with 369 *Vv* queens and 18 *Vc* queens.

2.2.4. Spermatheca size

The spermatheca was extracted from the abdomen using precision clamps (Dumont, 55I). The spermatheca was then placed in a drop of Ringer's solution on a microscope slide. Photographs were taken using a camera (CAMEDIA C-7070) at magnification ×6 (Fig. 2). The diameter of the spermatheca reservoir (Fig. 2AB a) was measured using the ImageJ1® image processing Software. In order to avoid a year effect, when comparing the body weight and the spermatheca diameter of the two species, we applied a correction factor, obtained by dividing the spermatheca diameter by the head width of the queen. For six specimens of *V. crabro*, we were unable to take good enough photographs of the spermatheca, and thus only 48 queens of this species were used for this part of the analysis.

2.2.5. Sperm count

After removing the external spermatheca envelope, the spermatheca content was spread in a drop of Ringer's solution by pressing it with clamps. The sperm fixation method described for parasitoid wasps by Bressac and Chevrier (1998) was applied. This method consists of a homogenization of the mix by clamp rotation, ethanol fixation, and DNA staining using 4′-6-diamidino-2-phenylindole (DAPI). This method has previously been used for counting sperm in seminal vesicles and for a description of spermiogenesis in male *V. velutina* (Poidatz et al., 2017). Having initially counted sperm five times on two slides, in 5, 10, and 15 microscope fields, we decided to count sperm in 10 microscope fields (Average spread = 25%). We counted all the visible sperm nuclei, except those in which half of the nuclei was missing. Although this led to reduced numbers, such technique prevented sperm loss or destruction during successive manipulations. For four urban *V. velutina* queens, we were unable to perform a sperm count. Sperm length was assessed in 15 randomly selected individuals in each species. In total, the sperm and nuclei lengths of 130 spermatozoa of *V. velutina* and 150 spermatozoa of *V. crabro* were measured from photographs (magnification ×100) using ImageJ1® software.

2.3. Statistics

Results are presented as the means ± SD. Analyses were performed using R 3.2.2 statistical software. A Shapiro test was used to assess the normality of the data. To assess the strength of the explanatory variables, we performed an ANOVA for the continuous variables or a GLM for discrete variables, with the fixed effects being 2015 or 2016, and the species (*V. crabro* or *V. velutina*). To compare sperm length, number, and morphological characteristics, a Student's *t*-test was used when the data were normally distributed; otherwise, a Kruskal–Wallis test was used. To examine the correlation between different parameters, we used a Pearson correlation test if the arguments had a normal repartition, and a Spearman correlation test if at least one argument did not. For the comparison of fat level between species, which was based on a visual scale, we used a Wilcoxon rank sum test.

In the comparison between species, a correction factor obtained by dividing by the head width was applied for the weight and the spermatheca diameter to avoid a year effect.

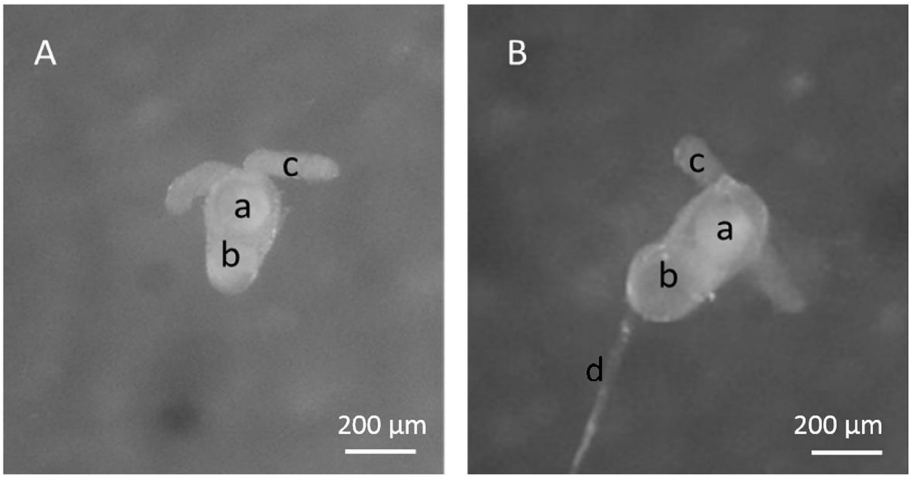

Fig. 2. Photographs of the spermatheca of *Vespa velutina* (A) and *V. crabro* (B) containing a condensed mass of sperm (a) in the reservoir (b), and the spermathecal accessory gland (c). The spermathecal duct is visible in *V. crabro* (d).

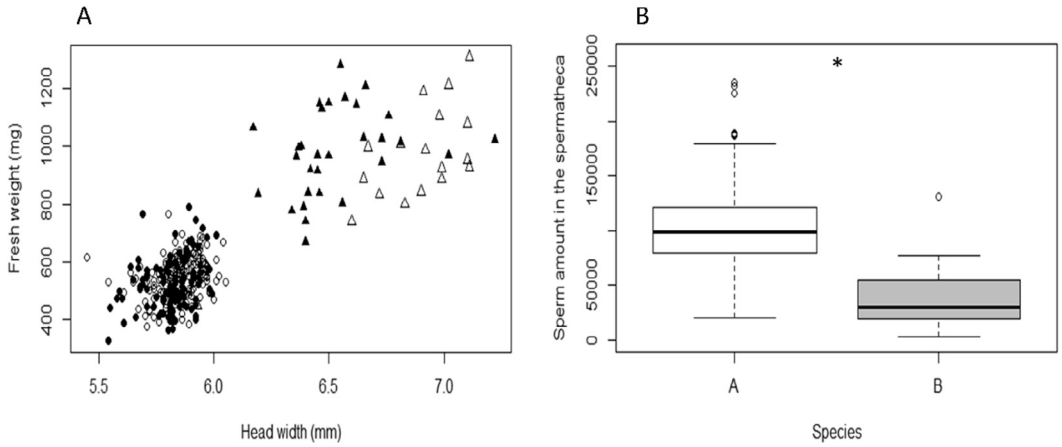

Fig. 3. (A) Head size as a function of fresh weight in the foundresses of *Vespa velutina* (circles) and *V. crabro* (triangles) in 2015 (white) and 2016 (black). (B) Total amount of sperm in spermathecae of the foundresses of *Vespa velutina* (A) and *V. crabro* (B) Kruskal–Wallis test $P < 0.05$.

The effect size was calculated using the Cohen's (d) formula, based on differences between means.

3. Results

3.1. Interspecific comparisons (means ± SD)

The head width was significantly smaller in *Vv* than that in *Vc* (respectively mean ± SD: 5.83 ± 0.09 mm and 6.66 ± 0.27 mm; *t*-test, $p < 0.0001$, $N = 237$, $d = 1.80$). For both species, individuals heads were smaller in 2016 (*Vv*: 5.82 ± 0.09 mm and *Vc*: 6.53 ± 0.22 mm) than in 2015 (*Vv*: 5.84 ± 0.09 mm and *Vc*: 6.90 ± 0.16 mm) [*t*-test $p = 0.01$ for *Vv* ($N = 184$, $d = 0.33$) and $p < 0.001$ for *Vc* ($N = 53$, $d = 1.11$)]. The queens of *Vv* and *Vc* weighed 540.38 ± 77.48 mg and 985.12 ± 150.71 mg, respectively (significant difference: *t*-test, $p < 0.001$, $N = 237$, $d = 2.33$). See Fig. 3a for an illustration.

The spermatheca diameter corrected by the head width of *Vv* queens (0.09 ± 6.10^{-3} mm, $N = 184$) was significantly lower than in *Vc* ($0.10 \pm 5 \times 10^{-3}$ mm, $N = 48$), (*t*-test, $p < 0.001$, $d = 0.28$).

The amount of sperm in *Vv* spermathecae ($111.56 \pm 29.65 \times 10^3$ sperm) was significantly higher than that in *Vc* ($48.26 \pm 19.19 \times 10^3$ sperm), (Kruskal–Wallis test: $k = 123.74$, $p < 0.001$, $N = 237$, $d = 1.58$) (Fig. 3.b).

The average spermatozoa length of *Vv* (122.17 ± 19.99 µm) was smaller than that of *Vc* (201.68 ± 26.86 µm) (Kruskal–Wallis test: $k = 206.26$, $p < 0.001$, $N = 280$, $d = 1.72$). Sperm nuclei length was, respectively, 12.31 ± 1.30 µm and 14.61 ± 1.25 µm [*t*-test: $p < 0.001$, 95% CI (1.992; 2.594), $N = 280$, $d = 1.34$].

The fat level (category) of *Vv* queens (2.78 ± 0.67) was significantly lower than for *Vc* queens (3.43 ± 0.61) (Wilcoxon rank sum test, $p < 0.001$, $N = 204$). A strong correlation was found between foundress fresh weight and fat level (Spearman test, $p < 0.001$). The fat level did not change with time in *Vv* queens during the sampling period ($R = 0.072$, least square, $t = 0.07$, $p = 1$, $n = 185$). Moreover, when we compared the fat level of *Vv* queens trapped in the first month in 2015 with that of *Vc*, we again observed a lower fat level in *Vv*, with less variability (Wilcoxon rank sum test with continuity correction: $W = 2176.5$, $p < 0.001$, N*Vv* = 53, N*Vc* = 48).

Among the ovaries dissected from 53 Vc foundresses, we observed 50 ovaries with 16 ovarioles and three ovaries with 14 ovarioles. We observed 16 ovarioles in all the ovaries dissected from 184 Vv foundresses. Ovarian development began approximately 15 days earlier in *Vv* than in *Vc*. Furthermore, *Vv* queens carrying three eggs ready to be laid in their ovarioles were found one month earlier than *Vc* queens (Supplementary data, Table 2). At the end of the sampling period, we observed a maximum of seven eggs in *Vv* ovarioles, but a maximum of only three in *Vc*. The maximum egg production in the ovarioles of *Vv.* queens was observed in the second half of April in spring 2015, and at this time, yellow bodies were visible in the ovarioles of some *Vv* queens, indicating that they had begun to lay eggs. In *V. crabro* the yellow bodies appeared more than one month after (Supplementary data, Fig. 1).

3.2. Correlations between the different parameters

The data obtained from correlation matrices for the two species are summarized in Table 1. In *Vv*, we observed significant correlations between head width and weight, that last being correlated with spermatheca diameter, this last strongly correlated with sperm content. In *Vc*, a significant correlation was observed between head width, weight, spermatheca diameter and sperm content. To conclude in both species, we observed a correlation between weight and head width, and a significant correlation between spermatheca diameter and sperm content in the spermatheca.

Table 1
Correlation matrix of the different parameters observed in foundresses of the hornet species *Vespa velutina* and *V. crabro*. P – value in white. rho/cor value: in grey. Abbreviations: Head = head width, Weight = fresh weight, spt diam = spermatheca diameter, sperm = amount of sperm in the spermatheca. p = Pearson correlation test, s = Spearman correlation test.

		***V. velutina* (N = 289)**				***V. crabro* (N = 53)**			
	>Rho / cor	Head	Weight	spt diam	sperm	Head	Weight	spt diam	sperm
p-value	**Head**	-	0.306 p	**0.028** p	**0.107** s	-	**0.398** p	-0.29 p	0.445 s
	Weight	**1.12x10^{-7} p**	-	0.164 p	**0.06** s	**0.005** p	-	**0.178**p	**0.123** s
	spt diam	0.634 p	**0.006 p**	-	-0.204 s	**0.047** p	0.224 p	-	- 0.327 s
	sperm	0.0684 s	0.298 s	**0.0006 s**	-	**0.001 s**	0.401 s	**0.02 s**	-

4. Discussion

The *Vv* queens were 79% lighter than *Vc* queens and their head width was on average 18% smaller, which is consistent with the species descriptions by Linnaeus (for *Vc*) and Lepelletier (for *Vv*). For both species, all the queens captured during spring were found to be mated. The spermathecae of the two hornet species observed here had the same approximately spherical shape, and differed only in size, being 16% larger in *V. crabro*, which is a very similar proportion compared with this insect's total size. This is consistent with the known morphology of spermathecae in Vespids (Martins et al., 2005). The sperm morphology was very similar in the two *Vespa* species, being thin, elongated, and slightly curved, which is consistent with the observations of Mancini et al. (2009). Compared with the sperm of *Vc*, those of *Vv* were 65% shorter with an 18% shorter nucleus, but were 43% more numerous. Initiation of egg production and oviposition were one month earlier in *Vv* than in *Vc*. *Vespa crabro* had higher fat reserves with a smaller amount variation than *Vv*, in correlation with its total weight.

In addition to outperforming *Vc* in behaviours potentially related with nest initiation (Monceau et al., 2015b), our study demonstrates that *Vv* also outperforms *Vc* in egg production timing. However, it should be noted that we measured egg production at the beginning of the cycle during the sampling period (for illustration, a detailed description of the development cycle of *Vespa affinis* is presented by Martin, 1991), and thus the foundresses had not attained their maximal egg production rate. Accordingly, we are unable to predict the final egg production based on the measurements made in the present study. Furthermore, the yellow bodies observed in the ovarioles of *Vv* queens are evidence of oviposition, but cannot be used to determine the number of egg laid since some eggs could be eaten after been laid (Cini et al., 2014). The fate of egg maturation should be linked to nest foundation. As mentioned in the introduction, a significant proportion of the mature *Vv* nests are found in relatively cryptic sites, and are thus potentially suitable for *Vc*, and a large proportion of the *Vv* nests are initiated in such sites before relocation. Competition during this critical period would appear to be plausible. As the number of produced gynes is considerably higher in *Vv* compared with *Vc*, (spring trapping, Monceau et al., 2013), this would enhance the potential efficacy of such occupation. The time differential in the reproductive cycle of *Vv* and its high fecundity could therefore be advantageous to this species in terms of founding and defending nest sites. Indeed, the timing of worker production is linked to the timing of colony foundation: 'first come first served'. The workers will defend the nest, explore, and locate resources with very limited competition during this initial period, which is a key factor for potential colony expansion. The early production of *Vv* workers could thus be advantageous in terms of colony defence against the larger *Vc* queens at the beginning of the colony cycle, the latter of which could attempt to usurp nests already founded by the congeneric *Vv*. Although interspecific usurpation of colonies between *V. velutina* and *V. crabro* has yet to be reported, it is not so rare in vespid species (Spradbery, 1973; Edwards, 1980; Matsuura and Yamane, 1990; Cervo et al., 2004).

In addition to local exclusion for nest initiation where the two species are present, which may involve a substantial proportion of the *Vc* foundresses, there may also be temporal exclusion. Accordingly, to counter the aggressiveness and strength of *Vc*, *Vv* have developed a strategy based on rapid reproduction of large numbers of individuals. The high precocity of *Vv* in its ovarian function and the larger stock of sperm allow this species to produce more workers early, which can compete with other vespids for resource collection. Numerous cases of aggressive interaction have been observed between *Vc* and *Vv* workers gathering pieces of tree leaves (D. Thiéry, pers. Obs.). This precocity of *Vv* may also be advantageous with respect to feeding on bee colonies. For example, when hunting as a group, *Vv* workers could benefit from rapidly reducing beehive defences, thereby ensuring a longer and safer hunting period. The high quality of such resources would undoubtedly contribute favourably to the number and quality of the next generation of *Vv* reproducers, as has also been hypothesized by Matsuura (1988).

Our finding of a lower variation in the levels of fat reserves in *Vc* compared to *Vv* could be biased by the lower number of sampled queens in this species (N = 54 *Vc* vs. N *Vv* = 183). However, we can assume that during initiation period, the fat level is a critical parameter amongst the life traits of the foundresses. Low fat level could have several repercussions, including a lower number or size of eggs, a higher requirement for sugar at this period, and less resistance to climatic variation (Strohm, 2000; Toth, 2005; Weissel et al., 2012). A paucity of fat in *Vv* could be compensated by the production of a higher number of gynes compared with *Vc*. The fact that the fat level did not vary with time in the captured *Vv* queens could suggest that the queens with fat reserves lower than level 2 in our analysis did not survive through winter, possibly explaining why we found only 2.65% of the dissected *Vv* foundresses in this case. Alternatively, it could indicate that in this environment, the queens of *Vv* are able to maintain or enhance their fat level above the level 2 in our scale (*i.e.* there is sufficient rich resources available).

Recently, Kovacs et al. (2012) suggested that 'mating should not adversely affect female viability in social insects'. We can thus assume that a large majority of the queens in these species are fertilized in the autumn, or that unfertilized gynes do not overwinter. To date, *Vv* matings *in natura* in Europe have all been observed at the end of autumn, on the ground adjacent to nests (J. Poidatz, O. Bonnard pers obs) or on the ground under or adjacent to nectariferous plants (J. Poidatz, pers obs.; K. Monceau pers com.). In a recent study Wen et al. (2017) described *in natura* mating in different subspecies of *V. velutina* in China: the gynes were able to mark a spot with pheromones which attract males, creating a Male Congregation Area (MCA). This behaviour has not been observed yet in the invasive *V. velutina var nigrithorax* in Europe, neither in *V. crabro*.

The observation that there was two times more sperm in the spermathecae of *Vv* than in those of *Vc* is consistent with the following observations. (1) Population size: colonies of *Vv* are considerably larger than those of *Vc*, with 15,000–30,000 individuals annually produced in *Vv* colonies and 700–1400 individuals produced in *Vc* colonies (Nadolski, 2012; Rome et al., 2015). (2) Mating number: multiple mating occurs in *Vv* as demonstrated by Arca (2012). There are on average 2.4 matings per gyne in *Vv*, (a maximum of 8 in France), and the first queen introduced into Europe was mated in China by at least 5 males prior to its introduction (Arca et al., 2015). In contrast, only approximately 1.1 mating per gyne have been described for *Vc* (Foster et al., 1999; Spiewok et al., 2006). (3) The spermatozoa of *Vv* are 0.65 times shorter than those in *Vc* and are more compacted within the reservoir. Compared to spermatozoa stored by females, *Vv* males had sperm in large excess (Poidatz et al., 2017). Sperm could exhibit morphological variations as a consequence of selective constraints on male paternity by sperm competition (Wedell et al., 2002; Snook 2005) due to frequency of female multiple matings. The shorter sperm in *Vv* is consistent with selection of high concentrations in ejaculates, whereas the longer *Vc* sperm would be less constrained by numbers because females typically mate only once. The multiple mating observed in *Vv* foundresses is a particular characteristic compared to other *Vespa* species (Strassmann, 2001; Cole, 1983), and is a very useful life trait for an invasive species, as illustrated in the case of the single queen introduced into France (Arca, 2012). Multiple mating in *Vv* could be a strategy to ensure both high potential fecundity and general brood genetic diversity, as has been observed for other social hymenopterans (Page and Metcalf, 1982; Cole, 1983; Crozier and Page, 1985; Ross, 1985; Ratnieks and Visscher, 1989; Keller and Reeve, 1994; Boomsma and Ratnieks, 1996; Schmid-Hempel and Crozier, 1999). The correlation observed between the weight and the spermathecal content in both *Vespa* species is interesting, because it could suggest that heavier gynes could in some way optimize their mating(s), which has been observed in fruit flies (Blay and Yuval, 2014), or they may prove more attractive

to potential mates. This last hypothesis has been demonstrated in moths (Xu and Wang, 2009). Compared with *V. orientalis* queens which have average 1.486 millions sperm stored at spring (Elbassiouny, 2007), *V. velutina* and *V. crabro* store 10 time less sperm in their spermathecae. This could be related with the nest population of those species.

The findings of this study bring to light several common traits but also differences in the fertility potential and fecundity of the queens of the endangered European hornet *V. crabro* and those of the invasive Asian hornet *V. velutina*. *Vv* queens are earlier in preparing eggs than *Vc* queens, and also have shorter but twice the number of sperm contained in a smaller spermatheca compared with *Vc*. The precocity and high fertility of *Vv* queens probably favours it over *Vc,* not only in terms of potential local exclusion during nest initiation, but also *via* temporal exclusion for territory and resource defence. The number, physiology, and precocity of *Vv* foundresses may have helped this species to compensate for its smaller size compared with the congeneric *Vc,* which is confirmed by Cini's et al. (2018). All these differences could explain the high brood production in *Vv*, and thus the high number of workers able to predate on bee hives. They also have important implications for the production of gynes in subsequent generations. Collectively, these observations might explain the observed supremacy of *Vv* over *Vc,* and hence the rapid colonization of this invasive species across European countries.

Acknowledgements

We thank all the beekeepers of the Bordeaux area, Jean Paul Croce, Annie Manon, Claude Berry, Claude Delfau, and Yves Goteau, who assisted in the collection of hornet queens. Dr Rita Cervo and one anonymous reviewer are acknowledged for valuable comments on the manuscript. We thank Bayer Crop Science, represented by Dr. Bénédicte Laborie, for financial support to the first author. We also thank our native English-speaking colleague Avril Wellwood-Foucard for English language corrections.

5. Disclosure

The authors declare no conflict of interest. The funding sponsor of the first author funding had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jinsphys.2018.07.004.

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