Mate-searching behavior in the invasive German wasp, *Vespula germanica*, in Patagonia

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Abstract

In order to increase the probability of reproduction, social insects can adopt various mate-finding strategies, such as increasing densities of males at specific locations, and/or visual and chemical cues that attract the opposite sex. In field and laboratory studies we investigated strategies used by the invasive eusocial wasp *Vespula germanica* (Fabricius) (Hymenoptera: Vespidae). In tethered flight assays, we established contrasting flight patterns in females and males that may partly explain how related individuals distribute spatially during the mating period. We also determined experimentally, in the field and in the laboratory via olfactometer assays, that gynes produce airborne pheromonal cues that attract drones and are important during mate location. Our field trials also suggest that visual cues play a role in mate location. We conclude that in addition to aspects of the social biology of the species, an efficient mate-location strategy can partly explain the invasion success of the species. Tools to mitigate the damage caused by yellowjackets may be developed by focusing on reproductive castes, in addition to workers.

Introduction

A good understanding of how populations expand is important for managing biological invasions. It is widely known that the invasion process can be divided into three successive stages: 'arrival', when individuals move into new areas outside their native range; 'establishment', when populations grow sufficiently that extinction is unlikely; and 'spread', when populations expand their range in new areas (Liebhold & Tobin, 2008). The dynamics of this process depends strongly on the dispersal capabilities of individuals, in addition to the species mating behavior (Kokko et al., 2014). Parameters relevant to a certain mating system might include the number of times individuals mate, the degree of relatedness with mates, how mates are found and chosen, and which sex searches at a specific moment of the process, in addition to population density. Many of these factors will interact and can have strong ecological

and evolutionary implications as ultimately they affect fitness and invasion capacity of a given population (Kokko & Rankin, 2006; Kokko et al., 2014).

The German wasp, *Vespula germanica* (Fabricius) (Hymenoptera: Vespidae), is a eusocial hymenopteran native to Eurasia and Northern Africa that in the past decades has invaded many parts of the world (Beggs et al., 2011), including the Americas. The species was first recorded in Argentina in 1980 (Willink, 1980), in northwestern Patagonia, and since then it has spread across the region and is now well-established in many areas (Masciocchi & Corley, 2013). The overwintering of mated queens in human goods, the absence of natural enemies, their outstanding behavioral plasticity (e.g., opportunistic foraging and nesting behavior), and their close association with human settlements have all been suggested to be key drivers of invasion success (Crosland, 1991; Farji-Brener & Corley, 1998; Beggs, 2001). Generally, in temperate regions the annual cycle of a colony is as follows. In early spring, a single mated queen starts a new colony, slowly building up worker numbers, reaching up to thousands of individuals in late summer. With lower temperatures and shortening

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days in autumn, gynes and drones (female and male reproductive individuals, respectively) emerge from nests to mate. Shortly after the reproductive caste leave for their mating flights, workers still within the colony and drones die. The mated females are the only caste to overwinter, which they do in sheltered places. The cycle continues the following spring with the establishment of a new colony (Spradbery, 1973a; Edwards, 1980). Especially in invaded regions, yellowjackets can negatively impact industries, society, and the natural environment (Beggs et al., 2011; MacIntyre & Hellstrom, 2015). Despite the species pest status in many parts of the world, management tools are limited and current population control strategies rely on reducing the number of established nests using traps containing toxic baits targeted at workers, in addition to manually destroying nests (Beggs et al., 2011). The extent to which these mitigation strategies work, is highly variable and normally do not suppress the problem. Population management through the behavioral manipulation of reproductive castes has not yet been targeted (as a pheromone acting at long range is still unknown); still, this strategy could offer additional and valuable mitigation tools (Foster & Harris, 1997; Rodriguez-Saona & Stelinski, 2009).

The reproductive behavior of social vespids has not been intensively studied, probably because of the inherent difficulty in studying such systems due to the fact that the mating period is short and individuals are elusive. Some of the existing observations report that drones emerge from parental nests ahead of gynes and congregate in specific areas, sometimes around shrubs and trees and wait for females to arrive (Spradbery, 1973b; Post, 1980; Greene, 1991; AS Martínez & M Masciochi, pers. obs.). Previous research has also indicated that *V. germanica* gynes, as well as other social vespids, can be polyandrous, with a study reporting that more than 85% of gynes mate multiply with unrelated drones (Ross, 1983; Goodisman et al., 2002; Loope et al., 2014). Additionally, and as expected, pheromones are involved in mate location in this group of insects. Two types of pheromones have been described in social vespid wasps: a gyne-produced pheromone to attract males and stimulate copulation (Reed & Landolt, 1990; Brown et al., 2013; Derstine et al., 2017) and a male-produced pheromone thought to be relevant in territory and perch marking (this last one is found in many polistine species but not in *Vespula* spp.; Ayasse et al., 2001). Gynes of *Vespula vulgaris* (L.) and *Vespula squamosa* (Drury) have been found to attract drones via a volatile chemical compound, and cuticular hydrocarbons mediate gyne recognition by drones in the short range (Reed & Landolt, 1990; Brown, 2013; Brown et al., 2013). Despite the considerable amount of information on

V. germanica, mostly centered on worker biology and behavior, much of its mating system is not yet understood. Aspects that still need to be elucidated are, for example, the pheromones involved in mate location, which sex produces them, the relevance of visual cues, the movement of reproductive individuals in relation to their nest and each other, and nest emergence patterns.

The aim of the present study was to investigate behavioral patterns involved in the mate-searching process in *V. germanica* gynes and drones in Patagonia. We used an experimental approach, both in laboratory and field conditions, under the overarching hypothesis that, in order to maximize searching efficiency and in consequence the rate of mating encounters with unrelated individuals, movement associated to finding mates will not be random and will be directed by multiple factors. We performed three experiments: (1) in tethered flight mills in the laboratory we tested the flight capabilities of gynes and drones, impacting how they distribute spatially in the field; (2) via traps baited with live gynes and/or drones in the field, we investigated whether visual cues, in addition to a pheromonal compound, mediate the attraction response, ultimately increasing the rate of mate encounters; and (3) in the laboratory we corroborated one of the field-test results that suggested that a volatile pheromonal compound mediates the attractive response of drones toward gynes.

Materials and methods

Insects

Individuals from reproductive castes were obtained from dug-out nests removed from two locations 10 km apart, during the onset of the reproductive flight period (i.e., the end of the *Vespula* colony cycle) in the austral autumn (May 2015 and 2016) in the region of Bariloche, Patagonia, Argentina. Subterranean nests were anesthetized with ethyl ether (98% purity; Sigma Aldrich, St. Louis, MO, USA), excavated and immediately taken to the laboratory. Combs with operculated brood were placed inside mesh cages (30 × 40 × 10 cm) and left in controlled-temperature cabinets at L16 (24 °C):D8 (18 °C). Newly emerged reproductives were removed daily and kept in a second cabinet in groups of ca. 50 individuals in mesh cages (10 × 10 × 10 cm) under the same light and temperature regime with ad libitum water, honey, and pollen. Workers were not removed from rearing cages with combs. Reproductive individuals were used in bioassays when they reached the age of 9 ± 2 days. This criterion was based on previous studies on *Vespula maculifrons* (Buysson), that indicate flight occurs when individuals are sexually mature (Ross, 1983), in addition to preliminary

trials in our laboratory (unpubl.) showing that *V. germanica* individuals are capable of flight as of 7 days old.

Tethered flight assays

Tethered flight experiments were carried out in flight mills following the method detailed in Masciocchi et al. (2018). Drones were tethered to mills and left to fly for 24 h. The total flight distance was recorded for 78 drones and compared with previously published tethered flight distances of 41 unmated pre-hibernated queens (Masciocchi et al., 2018).

Field trials

Field experiments were carried out in order to determine the sensory modalities involved in *V. germanica* mate-finding behavior and evaluate differences between reproductive castes. A total of 42 traps with seven treatments were set up in the region of Bariloche, Argentina, between 13 April and 13 May 2016. Traps consisted of 1.5-l carbonated beverage plastic bottles cut in half with the lids removed and the top-half inverted and inserted into the bottom one. Two of these units were joined by two 15-cm wires so they would hang one on top of the other (Figure 1). Water (100 ml) and a drop of neutral detergent was added to the bottom unit. The baits were hung between the two bottles from a 15-cm wire hanging from the top. The treatments were designed to establish which caste and sensory modalities [i.e., chemical (volatile), visual, or both] were involved in the species mate-searching behavior. The treatments consisted of: (1) a control treatment with no visual or chemical stimuli; (2) chemical treatment with drones: four live drones contained in individual non-see-through mesh (0.5 mm) cylindrical cages (3 cm diameter, 5 cm long); (3) chemical treatment with four gynes, as in (2); (4) visual treatment with four dead drones (previously washed in alcohol, water, and ethanol to remove possible odor cues) hanging from a thin wire; (5) visual treatment with four gynes, as in (4); (6) both treatments with four live drones inside individual see-through mesh (5 mm) cages (2 × 2 × 5 cm); and (7) both treatments with four live gynes, as in (6). For those treatments that used live individuals as baits, when found dead they were renewed by live ones during the trap revision.

Live wasps used as baits were obtained in the same way as those individuals used in laboratory bioassays. Each treatment was replicated 6× (i.e., six traps/treatment) and positioned in a 15-ha uninhabited area in the region of Bariloche with high wasp density. Traps were hung randomly from branches at ca. 1.5 m high, each one separated from the closest one by at least 50 m. Traps were checked at weekly intervals and captured *V. germanica* drones and gynes were removed from traps at each revision.
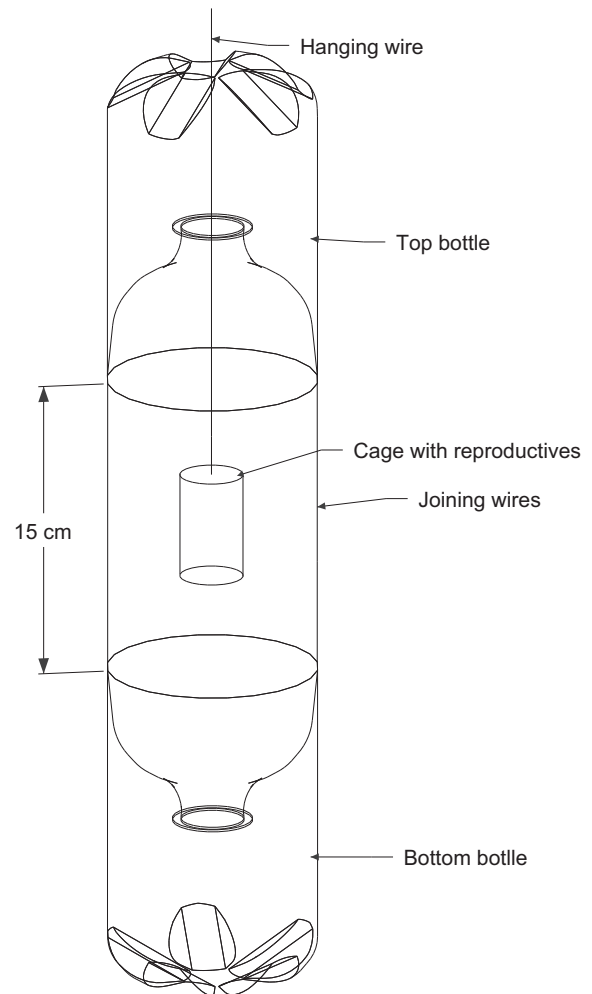

Figure 1 Trap used to evaluate the sexual attraction behavior of *Vespula germanica* reproductive caste and the sensory modalities involved in the process.

Olfactometry

The olfactory response toward individuals of the opposite reproductive caste was measured in a four-way olfactometer (Figure 2). The arena, made of polyamide (Grilon; Ems-Chemie, Domat/Ems, Switzerland), consisted of a central area and four symmetrical arms, based on Pettersson (1970) and Vet et al. (1983). The air flowed through the odor chambers, into each of the four arms and lastly through the central exit circular aperture at 0.5 l per min per arm. Air was previously filtered in 0.5 kg of activated charcoal and then humidified through 0.7 l of distilled water to prevent biases due to differences in humidity of odor sources (Martínez & Hardie, 2009). The arena was illuminated with white-light LEDs (2.5 m of LEDs strip set in a circumference at 1.5 m over the
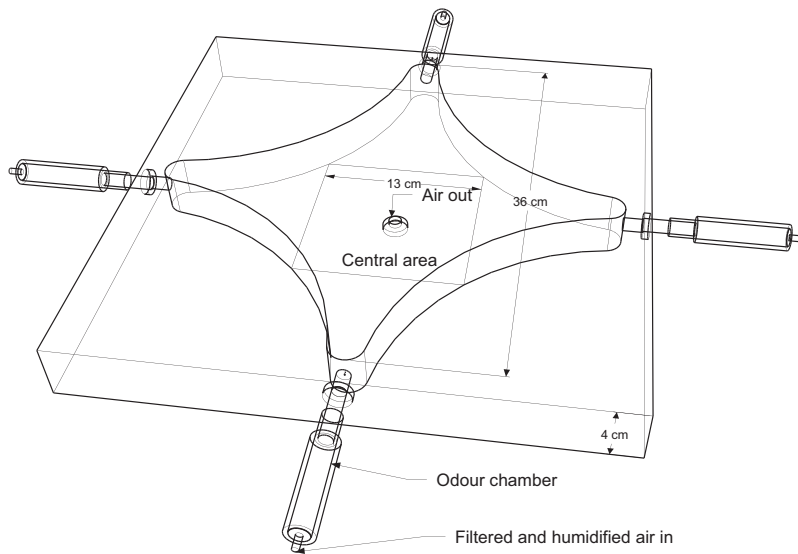
olfactometer with 60 surface-mounted diodes per m (60 W; Alic, Buenos Aires, Argentina). Individuals used in the bioassays were reared as described above.

Odor sources were placed in four individual glass chambers connected to the air stream that entered each of the olfactometer arms. The setup was used as a two-way olfactometer, as two odor sources were presented simultaneously to individual wasps. The response toward sources of clean air and the opposite castes was tested for 28 gynes and 28 drones. A single wasp was introduced in the central arena of the olfactometer and left for 1 min to acclimate after which the behavior (i.e., movement) was registered for 10 min. Drones were presented with two empty (i.e., clean air) glass cylindrical chambers (2 cm diameter and 5 cm long, not visible from inside the olfactometer) and two chambers containing a single unfertilized gyne that could move freely. Gynes on the other hand, were presented with two empty chambers and two chambers with one drone each. After each replicate, the olfactometer was washed thoroughly with water and alcohol and left to dry. After every four replicates, odor sources were renewed and in order to minimize orientation biases, their positions relative to the olfactometer arms changed according to a predefined rotation scheme. Opposite castes were obtained from colonies of at least 10 km apart to minimize relatedness (Goodisman et al., 2001).

Wasp movement during the assay was registered using a web camera (Genius FaceCam 1000; KYE Systems, New Taipei City, Taiwan) using the .avi file format at 5 frames s^{-1}. The position (X,Y coordinates) of the wasp was determined in each frame via analysis with ImageJ (Rasband et al., 1997–2016) in combination with the wrMTrck plugin (Nussbaum-Krammer et al., 2015). Once

the wasp position was determined in each frame, the percentage of time spent in each stimulus area (i.e., clean air vs. wasp) was determined (the time spent at the central area – defined as a square of 13 cm centered in the olfactometer – was excluded from the analysis).

Data analysis

All analyses were performed using R software v.3.3.2 (R Development Core Team, 2016). Tethered flight results were compared with the non-parametric Kruskal–Wallis test. Differences in frequencies (proportion of individuals) of flight distances were compared via a generalized linear model with an exponential distribution and a reciprocal link function. The proportion of individuals within each caste was used as the response variable and the interaction between caste and flight distance (m) was used as a predictive variable.

The numbers of individuals captured in each trap at each date (a total of 24 replicates per treatment) were compared via generalized linear models with a negative binomial distribution and a log-link function using number of captured individuals per trap per revision date as response variable, whereas the sensory modalities tested (i.e., chemical, visual, or both) within each bait-caste were used as explanatory variables. The goodness-of-fit of the model was evaluated by inspecting the models' residuals, whereas pairwise comparisons were done using contrasts.

The responses of gynes and drones in the olfactometer were compared with the non-parametric Wilcoxon rank sum test. In order to rule out possible pseudoreplication effects (i.e., individuals used as stimuli were renewed and rotated every four replicates), two additional statistical comparisons were carried out: the first test compared the

response toward each of the seven groups of wasps (gynes or drones) used as stimulus, the second test compared the response toward each arm (1–4) regardless of the stimulus present in each of them.

Results

Tethered flight assays

Median flight distance of gynes (3 002 m/24 h, n = 41) was higher than that of drones (418 m/24 h, n = 78; Wilcoxon rank sum test: χ^2 = 31.15, d.f. = 1, P<0.001). The probability distribution of flight distance for each caste was fitted to an exponential function [probability = scale (growth rate × flight distance)], with the following parameter estimates for drones: scale = 2.9, growth rate = 0.001; for gynes: scale = 0.47, growth rate = 0.0002 (Figure 3). These distributions were found to be different (χ^2 = 4.94, d.f. = 1, P = 0.026). As an indication of this difference, 66% of drones fly less than 500 m, whereas 60% of gynes fly more than 2 500 m; only 2.8% of drones flew more than 2 500 m.

Field trials

In total 42 drones were recovered from baited traps (n = 168 traps per revision date) and two from control traps (n = 28 traps per revision date; Figure 4). No gynes were captured in the field traps. More drones were found in traps baited with gynes than those baited with drones (Z = 2.169, d.f. = 191, P<0.05). Only seven drones were recovered from traps baited with drones (visual treatment: two drones, in two traps), chemical treatment (two drones, in two traps), and visual + chemical treatment (three drones, in two traps), whereas two drones were captured in the control treatment (two drones, in two traps). These drone-baited traps did not capture more than control traps (Z = 0.414, d.f. = 95, P>0.05).

Traps baited with gynes captured a total of 35 drones (n = 96 traps per revision date). The number of captured individuals in the chemical treatment (seven drones, in four traps) and visual treatment (four drones, in four traps) did not differ from the control treatment (two drones, in two traps) (Z = 1.29 and 1.01, respectively; both d.f. = 95, P>0.05). More individuals were captured using the chemical + visual treatment (24 drones, in three traps) than in the control treatment (Z = 2.32, d.f. = 95, P<0.05), but not vs. the chemical or visual treatment (Z = 1.39 and 1.89, respectively; both d.f. = 95, P>0.05). The efficiency (number of traps with effective captures × 100/total number of traps) of control traps was 8%, whereas the efficiency of traps baited with visible and live gynes was of 17%.

Olfactometry

Drones spent more time in the branches of the olfactometer that bore female wasps than in the ones with clean air (72 vs. 28%; Z = 5.73, d.f. = 1, P<0.0001; n = 28; Figure 5). On the other hand, gynes spent less time in the olfactometer arms with drones vs. the arms with clean air (55 vs. 45%; Z = 2.42, d.f. = 1, P<0.05; n = 28). No biases due to pseudoreplication were observed for groups of individuals used as stimuli (gynes with drones as stimulus: χ^2 = 4.74; drones with gynes as stimulus: χ^2 = 6.59, both d.f. = 6, P>0.05), nor due to biases with respect to the olfactometer arms (gynes with drones as stimulus: χ^2 = 2.07; drones with gynes as stimulus: χ^2 = 1.38, both d.f. = 3, P>0.05).

Figure 3 Probability distribution of flight distances during a 24-h assay of *Vespula germanica* drones (n = 78) and gynes (n = 41). Data on gynes were obtained from Masciocchi et al. (2018). Flight distance data were fitted to the exponential function: probability = scale$^{(growth\ rate\ ×\ flight\ distance)}$.
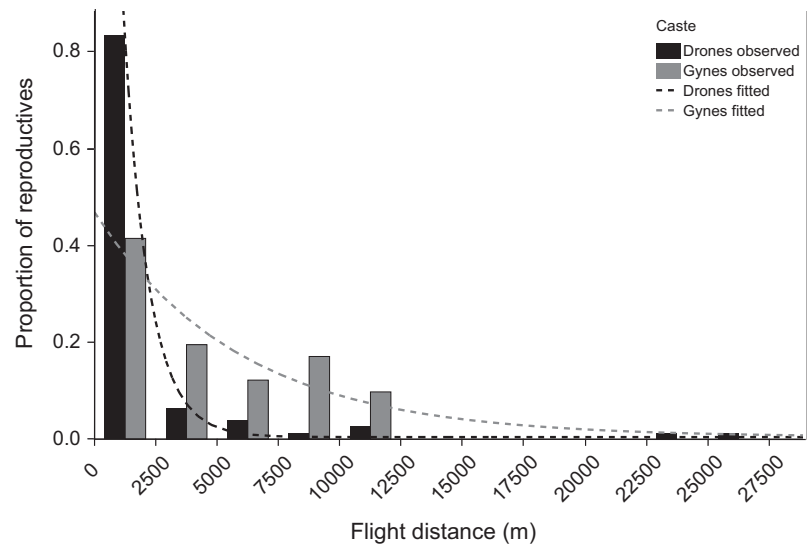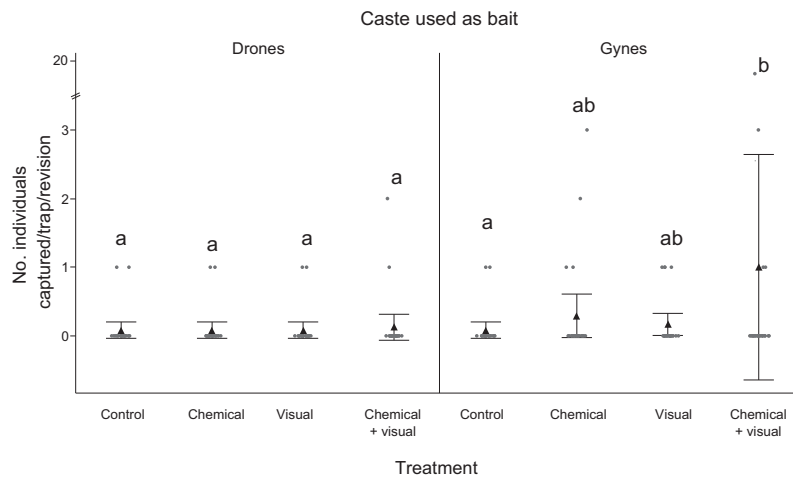
Figure 4 Mean (± 95% confidence intervals) number of *Vespula germanica* wasps captured in field trials during four revision dates using traps baited with one of seven treatments encompassing variations of neutral, visual, and chemical stimuli (n = 28 replicates per treatment; control treatment is represented twice for comparative purpose). Statistical comparisons were made within gynes or drones as bait (GLMs with negative binomial distribution and log link function); treatments capped with the same letter are not significantly different (P>0.05).
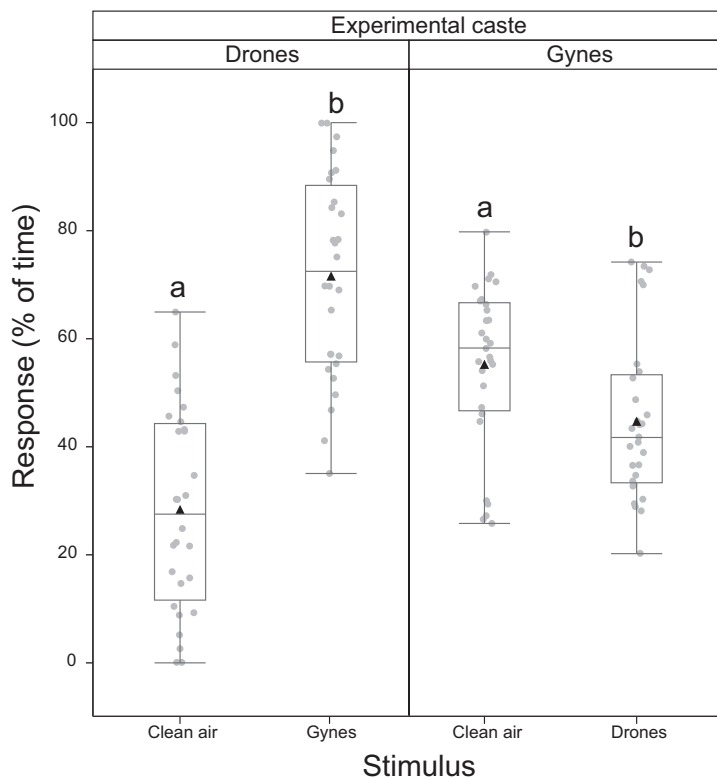

Figure 5 Olfactory response of *Vespula germanica* drones and gynes (both n = 28) in a four-arm olfactometer. Drones spent more time in arms containing gynes than in arms with clean air, whereas gynes spent less time in arms containing drones than in arms with clean air. Boxes indicate 50% of data spread, the line across indicates the median value, and the triangle the mean. Whiskers indicate the range of the data. Means within a caste capped with different letters are significantly different (Wilcoxon test: P<0.05).

Discussion

In this study we investigated behavioral mechanisms that could be involved in the mate location process of *V. germanica* reproductive castes. Our main results indicate that in tethered flight assays gynes have the capacity to fly, on average, 6× further than drones, whereas the frequency distribution of drone-flight is skewed toward smaller distances. We also observed that drones are attracted to gynes at various spatial scales, as demonstrated by the field results

and confirmed by the olfactometer assays. This attraction appears to be due to both chemical and visual cues.

Reproductive caste flight potential

In tethered flight assays we found a high proportion of drones with relatively low flight potential compared to that of gynes, and drones also had a broader flight-distance spectrum. This result was in line with the hypothesis that flight patterns of reproductive castes are different. As in other social insects, when reproductive castes initially emerge from nests, there is a high density of kin, and the difference in flight potential between drones vs. gynes seems an efficient and flexible mechanism by which related opposite reproductive castes are segregated, being beneficial at a population level by reducing inbreeding rates, especially when population sizes are small (Jaffé et al., 2009; Szulkin et al., 2013). In wild great tit populations, for instance, dispersal was found to be a widespread strategy by which inbreeding is reduced (Szulkin & Sheldon, 2008), as was found in eusocial Hymenoptera where females and males occupy the same nest (Tabadkani et al., 2012).

Our study suggests that differential flight patterns of reproductive castes could segregate related gynes and drones, consequently reducing inbreeding. Although there is no established link between tethered flight mill patterns and movement in the field, our results suggest that dispersal in *V. germanica* could be gyne-biased, with a higher proportion of drones remaining in the vicinity of the parental nest (66% of drones fly less than 500 m), whereas gynes have the capacity of flying larger distances (60% of gynes fly more than 2 500 m), but still a proportion of the gynes fly as little as drones. Although tethered flight assays are a simplification of what occurs in natural environments, it is an accepted method to compare movement potential of flying animals (Brodschneider et al., 2009; Bruzzone et al., 2009; Tosi et al., 2017; Masciocchi et al., 2018), especially useful in species where quantifying flight patterns in the field is difficult. It is also important to note that our tethered flight-mill studies are limited in the sense that flight potential was measured in the absence of potentially relevant information during the process of mate location, such as visual and olfactory cues, and abiotic factors such as natural light and wind. Adding these (presently unknown) relevant cues could change the flight patterns registered in the laboratory. Nevertheless, our results are useful for initial comparative purposes, and helpful in setting a baseline for future controlled experiments under more realistic scenarios. Ultimately, the conclusions from this study need confirmation with field studies assessing the behavior in a natural environment.

The observed differences in flight patterns in *V. germanica* reproductive castes would not only favor an increase in dispersal distances of gynes, but could also be a strategy that reduces chances of encounters with related mates. By doing so, inbreeding is minimized: most gynes would fly further than kin drone-aggregations, thus promoting outbreeding. Although drone flight potential is relatively low, it is important to note that a small proportion of them display long-distance flights (2.8% of drones fly more than 22500 m, which was more than any of the gynes), suggesting that these individuals could be important at increasing genetic variation in the longer term.

In addition to sex-biased flight patterns, pre- and post-copulatory mechanisms could contribute to the reduction in inbreeding in a species, such as polyandry, post-copulatory sperm selection, differential phenology of opposite castes at the moment of nest emergence, and intersexual kin discrimination. *Vespula germanica* queens have been reported to mate with more than one drone (Goodisman et al., 2002). This mating pattern could help reduce genetic bottlenecks at the early stages of invasion, as mated queens arriving into a new area would be mothers to half-sister gynes (Tregenza & Wedell, 2002; Cornell & Tregenza, 2007). Hence, genetic Allee effects (causing initial negative growth of low-density populations) arisen from inbreeding when populations are small (as happens in the initial stages of invasion) (Berec et al., 2007), might not be as strong as in other non-polyandric species. Intersexual kin discrimination is known to be a flexible trait in animals (Szulkin et al., 2013) and in hymenopterans it can range from preference for non-kin, via no preference at all, to preference for kin. For instance, whereas in *D. maculata* drones are attracted toward non-sibling gynes and avoid sibling gynes (Derstine et al., 2017), in the social paper wasp *Polistes versicolor* (Olivier) no mechanisms have been found to prevent mating with kin (de Souza et al., 2017). Previous research has also found indirect evidence that *V. germanica* queens do not mate with kin, as no relation was found between gynes and their mates in Australia, an invaded region where the species has been established for decades (Goodisman et al., 2002). Additional field observations (Spradbery, 1973b; Greene, 1991) suggest that intersexual kin discrimination could happen in *V. germanica*, but to our knowledge, no detailed behavioral study has been carried out yet.

Sex-biased dispersal of *V. germanica* reproductive castes could be an important factor in the observed invasion success, even though not proven by our study. Based on our results, we hypothesize that during the early stages of invasion, those gynes that display low flight potential remain near the parental nests and mate with related drones, whereas gynes with higher flight potential would not find mates. Later in the invasion process, with well-established populations, higher densities of reproductives, and new

individuals arriving into the area, queens with higher flight potential will mate with distant drone aggregations increasing the genetic variability. Future studies should be directed at evaluating the additional factors that could affect flight patterns of reproductive castes of *V. germanica*, taking into consideration more realistic scenarios, such as evaluating the effect of mating status, age, and the presence of same or opposite castes on flight potential. This will be essential to fully understand the species' mating system, its impact on inbreeding avoidance, and its invasion.

Sensory modalities used in mate location

Our field and olfactometer results both indicate an attraction of drones toward gynes at small and larger spatial scales, as well as the lack of attraction from gynes toward drones. The observed attraction of drones toward gynes in the field seems to be the result of the combination of volatile and visual cues, as only traps baited with visible live gynes captured more than control traps, whereas the olfactometer results confirm that airborne signals are involved in the attraction process. Even though traps treated with visible live gynes captured twice as much as control traps (8% of control traps captured individuals whereas the visual + chemical treatment captured 17%), the overall capture rate was low, with only a few traps responsible for the recorded differences. An explanation could be that male aggregations are rare, localized, and short-lived. Only traps positioned near eventual aggregations captured individuals. Future studies aimed at establishing how male aggregations are formed (e.g., their position vs. nest location, their genetic variability with respect to surrounding nests) should help to disentangle the intricacies of *V. germanica* mating behavior.

Our results suggest that an airborne sex pheromone is emitted by gynes, which attracts drones. Probably, once drones are close to the pheromone-emitting gyne, visual cues reinforce the attraction. Previous studies have found indications of volatile compounds emitted by gynes that attract drones in the closely related yellowjacket *V. vulgaris* (Brown et al., 2013), *Dolichovespula maculata* (L.), *Dolichovespula arenaria* (Fabricius) (Derstine et al., 2017), *Polistes exclamans* Viereck (Reed & Landolt, 1990), and *Vespa velutina* Lepeletier (Wen et al., 2017), but to our knowledge these studies did not test for visual effects, as the present field study did. Evidence on the relevance of visual cues in social hymenopterans is scarce. Recent studies indicate that this sensory modality seems to be of relevance in workers of the social wasp *Polistes dominulus* (Christ) at recognizing gender (Cappa et al., 2016), whereas queens of *Polistes fuscatus* (Fabricius) and *Polistes metricus* Say recognize opponent queens (Sheehan & Tibbetts, 2011). An additional study found that in *P. dominulus*, male ornaments are important in mating behavior showing that spot morphology functions as signal in both inter- and intrasexual selection (Izzo & Tibbetts, 2012). Although the context of these studies is not linked directly to mate location, they indicate that the use of visual cues has evolved in various contexts in paper wasps and could be a relevant source of information during mate location in *V. germanica*.

The olfactometer results also indicate that gynes avoid drones based on their smell, which could be part of the courtship or drone selection behavior displayed by the queen once in the close proximity of a drone. Previous studies report that during mating, *V. germanica* gynes are sometimes aggressive toward drones (Brown, 2013). As mentioned above, another factor that can result in gynes avoiding drones is a high degree of relatedness between male and female reproductives, as shown in *D. maculata* (Derstine et al., 2017). Nevertheless, during the experiment special care was taken to use unrelated gynes and drones in the same bioassay, hence the observed behavior cannot be the result of kin avoidance. We cannot rule out an artifact of the experimental design (e.g., visual or environmental cues missing), concluding that further research encompassing the location and acceptance behavior of both castes is needed.

This study contributes to our understanding of some of the mechanisms involved in the mating system of *V. germanica*. In the longer run this could be used to develop alternative techniques for managing undesired social wasp populations. The suggested sex-biased dispersal behavior could have important implications in the invasion process and guide the development of tools to ultimately encompass the manipulation of mating behavior in control strategies, by targeting drones (e.g., through mating disruption or mass trapping) with baited traps based on both chemical and visual cues and encompassing information about trap placement/spacing according to caste flight potentials.

Acknowledgements

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