Nest-departure behaviour of gynes and drones in the invasive yellowjacket *Vespula germanica* (Hymenoptera: Vespidae)

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Abstract

Inbreeding costs can be high in haplodiploid hymenopterans due to their particular mechanism of sex determination (i.e., single-locus complementary sex-determination system, sl-CSD), as it can lead to the production of sterile males. Therefore, mechanisms contributing to reduced inbred matings can be beneficial. In this sense, asynchronous nest departure of sibling drones and gynes could reduce kin encounters in social hymenopterans. Using six observation colonies, we determined under field conditions the nest departure behaviour of sibling reproductives of the social wasp *Vespula germanica* (Hymenoptera: Vespidae). We determined that sexuals leave the nests definitively and detected asynchronous departure not fixed to a particular caste at a seasonal scale in some colonies, as gynes or drones delayed their departure as a function of the departure of the opposite sex, depending on the colony. At a higher temporal resolution (i.e., within a day), we discovered that drones consistently began to leave nests 1 h before gynes and this difference was driven by those individuals that left on the same day as did the opposite-sex kin. Even though other mechanisms such as polyandry and differential dispersal could also be important at reducing inbred matings in the species, the observed departure patterns (i.e., in some colonies actually leave together with the opposite caste, while in others temporal segregation seems to occur) from nests could be complementary to the former and be important at reducing the negative effects of inbreeding in this invasive species.

Introduction

Mating with a close relative (inbreeding) is frequently undesired in sexual species as it can lead to the expression of deleterious, recessive alleles (Charlesworth and Willis, 2009). Particularly in haplodiploid hymenopterans, due to their particular mechanism of sex determination (i.e., single-locus complementary sex-determination system), the costs of inbreeding can be even higher, because sterile diploid males develop and reach the adult stage, representing an extra cost for the colony (Van Wilgenburg *et al.*, 2006; Tabadkani *et al.*, 2012). Many hymenopteran species display behaviours that reduce inbred matings, such as mating with more than one male (polyandry), kin recognition and differential spatial and temporal distribution patterns between sexes (Mazzi *et al.*, 2011; Loyau *et al.*, 2012; Pizzari and Wedell, 2013; Vitikainen *et al.*, 2015; Martinez *et al.*, 2018).

Eusocial insects share a communal nest where generations overlap and normally, during reproductive periods, the density of related reproductive individuals can be high around the nest. Thus, the timing of nest departure is critical to kin encounters (Ayasse *et al.*, 2001; Tabadkani *et al.*, 2012; Aguilera-Olivares *et al.*, 2015), therefore asynchronous departure patterns between relatives of the opposite sex could reduce the risk of inbreeding (Morbey and Ydenberg, 2001). Additionally, permanent nest departure (i.e., no return of reproductive individuals to the parental nest) would further help in reducing the rate of encounters with relatives since returning to the parental nest would involve recovering high densities of related individuals.

Vespula germanica is an eusocial wasp native to Europe, Asia and North of Africa, that in the last century has invaded forested and urban areas of New Zealand, Australia, South Africa, North America, Canada, Chile and Argentina (Lester and Beggs, 2019). In Argentina, the first detection was in 1980 (Willink, 1980) and since then, their populations have expanded considerably (Masciocchi and Corley, 2013). In invaded areas, this wasp represents a problem to a variety of human activities. For instance, honeybee hives can be lost due to wasps killing workers and stealing honey, thus impacting in reduced economic gains for beekeepers and in turn pollination efficiency in agriculture (Yeruham *et al.*, 2002; MacIntyre and Hellstrom,

2015). In temperate invaded regions, the wasp has an annual cycle in which mated queens overwinter and during spring, new colonies are established and start to develop. The population peak occurs during late summer, with colonies growing up to thousands of individuals, a time of the year in which they can become a significant threat. Despite its pest status, the tools available for population management are still limited. Currently, the toxic baits aimed at workers and manual destruction of nests are the main strategies used worldwide (Dimarco *et al.*, 2017; Lester and Beggs, 2019).

Targeting reproductive individuals (namely gynes and drones) is a promising alternative for control since it could potentially reduce the number of mating partners and hence colony density the following year. However, this is no simple task, since the reproductive behaviour of this social wasp is still largely unknown, especially when contrasted with the existing knowledge on worker biology and behaviour (Beggs *et al.*, 2011; Lester and Beggs, 2019). Some of the studies on reproductive behaviour in *V. germanica* indicate that mating occurs during the end of autumn, with field observations suggesting that drones emerge ahead of gynes and congregate in specific areas waiting for females to arrive (Spradbery, 1973; Post 1980; Greene, 1991). Gynes can mate more than once (polyandry), with pheromones and visual cues suggested to be involved in mate location (Ross, 1983; Reed and Landolt, 1990; Ayasse *et al.*, 2001; Goodisman *et al.*, 2002; Brown *et al.*, 2013; Loope *et al.*, 2014; Derstine *et al.*, 2017; Martinez *et al.*, 2018). Also, tethered flight bioassays indicate that movement is greater in pre-hibernated gynes than in drones, thus favouring spatial separation (Masciocchi *et al.*, 2016).

Given the relatively few reports addressing the reproductive behaviour of *V. germanica*, specifically those aimed at the possible patterns in nest departure of reproductive individuals, the aim of our study was to establish, under field conditions, the nest departure timing of sibling reproductives of *V. germanica*. Given that *V. germanica* gynes seem to not discriminate between kin and non-kin partners (Goodisman *et al.*, 2002), we hypothesize that asynchronous nest departure of sibling drones and gynes could contribute to reducing kin encounters, reducing, in turn, inbred matings.

Materials and methods

Study site

The study was conducted in the proximity of the city of San Carlos de Bariloche, Patagonia, Argentina (41°09′S, 71°18′W). Set at the foot of the Andes mountain range at an approximate elevation of 800 m, the region has a cool temperate climate with mean daily temperatures in winter (June–August) and summer (December–March) ranging between 2–3°C and 13–15°C, respectively (data from Instituto Nacional de Tecnología Agropecuaria, EEA Bariloche). In this area, *V. germanica* has an annual cycle, in which queens emerge from hibernation in early spring (September) and colonies reach their peak at the end of summer (March).

Observation nests

Twelve nests were excavated in gardens and vacant land, at the beginning of the *V. germanica* worker flight season (January and February) during 2 consecutive years (six in 2017 and six in 2018). Subterranean nests were anaesthetized with ethyl ether (98% purity; Sigma Aldrich, St. Louis, MO, USA), excavated and immediately placed in experimental boxes. Each experimental box consisted of an aluminium square box (30 cm per side) with a cylindrical entrance, to which a transparent plastic tube was added (25 cm in length × 2.5 cm diameter) to allow entry and exit of wasps. The top side of the square box was covered with a removable cover, under which a glass top was placed to observe colony development inside. All remaining sides were insulated with high-density Styrofoam™ (2.5 cm thick). Preliminary studies showed that wasps continue with their normal activities under such conditions after a few days (Masciocchi, unpubl. data). Experimental boxes were placed outdoors, under ambient conditions within IFAB (Instituto de Investigaciones Forestales y Agropecuarias Bariloche) grounds (−41°7′24″S, −71°15′5″W) where observations took place.

Wasp traffic observations

In each experimental colony, the inbound/outbound traffic of all individuals was registered by one observer every hour for 10 min between 09:00 and 17:00. Traffic observations started a few days prior to the onset of reproductive individuals commencing to depart from the nest, and observations continued almost every day (except for rainy days when wasp traffic ceased), until a week after the last reproductive had been observed to leave the nest. The temperature at the nest entrance was recorded at each 10 min interval using a digital thermometer. In order to obtain an indication of colony health, worker inbound and outbound traffic was also recorded during the same observation periods. Once reproductive departure period was over, nests were anaesthetized and the number of combs, cells and reproductives left inside quantified.

Data analysis

For each colony, the duration of departure periods for reproductive castes was compared via a Paired Wilcoxon Signed Rank test (pairing the departure duration of drones vs. gynes from the same nest). The traffic of reproductive individuals was analyzed at two different time scales: seasonal and daily. We compared departure of gynes and drones under the survival analysis framework (Kleinbaum and Klein, 2010) considering the moment of departure from the nest of each reproductive individual as the 'time to event'. For this, we used the Cox Proportional Hazards model to contrast the patterns between two different groups. The output includes a 'risk ratio' for the different groups, where values >1 indicate a higher probability of departure vs a reference (for example, a risk ratio of 2 is interpreted as twice the probabilities of departure compared to the reference group). Kaplan–Meyer survival curves are used as a graphical aid to illustrate the observed departure probabilities. All analyses were performed using R software v.3.6.1 (R Development Core Team, 2019).

Seasonal departure

Because the onset of the reproductive flight period was variable between nests and the objective of the study was to investigate the within-nest variation of gynes and drones, we standardized the departure date of each individual when the first reproductive (male or female) left each nest. With the date of departure normalized, we used Cox proportional hazards models with the number of days since the first reproductive individual left the nest as the response variable and year, nest and caste as main effects,

in addition to the interaction between caste and nest. Due to differences found in the departure patterns between nests, we reassigned a new a *post-hoc* explanatory categorical variable with 3 levels depending on the statistical significance of their departure order of castes. Castes were reassigned to (i) leaving early ($P < 0.05$), (ii) leaving late ($P < 0.05$) or (iii) leaving at the same time ($P > 0.05$).

Daily departure behaviour

The hour of departure of each individual was rounded to the nearest hour (remember, only 10 min for every hour were sampled for each nest). Overall median departure times were calculated and compared between castes with the non-parametric Wilcoxon Signed Rank test. A more detailed analysis was carried out to establish the degree of within-day variation between castes with Cox Proportional Hazards models as described above, with the difference that due to high variability between nests in the hour of departure, we used as the response variable the hours elapsed since the first individual left the nest that day. We additionally compared individuals emerging together with siblings from opposite caste on the same day vs those individuals emerging alone (i.e., without the siblings from the opposite caste on that day).

Results

Four of the nests excavated in 2017 and two of 2018 did not establish due to unknown reasons. Therefore, we used the six remaining nests (two nests in 2017 and four in 2018) for data analysis. During 2017, nests were monitored on 23 days of the 30-day departure period, while in 2018 the departure period lasted 36 days, out of which 33 days were surveyed. During both years, a total of 77 gynes and 261 drones were observed to emerge from nests, but no individuals were observed to return (table 1). 12 ± 3 (mean ± standard error) gynes and 40.3 ± 10.2 drones per nest were recorded emerging, with a mean sex ratio of 4:10 (gynes: drones, range 0.1–0.8). No reproductive individuals were found inside nests once the departure period was over (with the exception of the foundress queens). The onset of nest departures by reproductives for the first year was 5 April and during the second year, 31 March. The mean duration of the nest departure period (period of the first and last reproductive departing from the nest, averaged over both years) was 27 days. The mean departure period of gynes was of 15 days ± 4 (mean ± standard error, $n = 6$ nests), whereas for drones this period was significantly longer with a mean of 22 ± 2 days (mean ± standard error, $n = 6$ nests; $S = 10.5$, $P = 0.03$). No reproductive individuals were found within nests once the departure period was over.

Seasonal departure

No statistical differences in nest departure were registered between years ($\chi^2 = 0.18$, $P = 0.67$, d.f. = 1) or castes ($\chi^2 = 0.39$, $P = 0.53$, d.f. = 1), while a difference in departure was found between nests ($\chi^2 = 29.7$, $P < 0.0001$, d.f. = 5) (fig. 1). Because of the variability in departure patterns between colonies, a more detailed analysis of caste departure within nests was carried out, showing that opposite reproductive castes emerged according to one of three temporal patterns. In patterns 1 and 2, gynes emerged before drones (nests 16 and 23, $\chi^2 = 6.3$, $P = 0.01$, d.f. = 1, risk = 7.3 and $\chi^2 = 59.9$, $P < 0.0001$, d.f. = 1, risk = <0.001, respectively) and drones emerged before gynes (nests 8 and 182, $\chi^2 = 5.44$,

Table 1. *Vespula germanica* nests observed during 2 consecutive years with inbound and outbound traffic of all castes registered during the reproductive departure period at hourly intervals, during 10 min per nest from 09:00 to 17:00 h

Year	Nest			Gynes				Drones				Gynes & Drones	
	Identification number	Worker cells	Queen cells	Number of individuals	Date first individual left nest	Date last individual left nest	Emergence duration (days)	Number of individuals	Date first individual left nest	Date last individual left nest	Emergence duration (days)	Sex ratio (G/D)	Emergence duration (days)
2017	8	2459	295	12	23/04	05/05	12	48	05/04	03/05	28	0.3	30
	18	4077	300	18	08/04	05/05	27	76	05/04	03/05	28	0.2	30
	16	2890	183	5	12/04	18/04	3	10	12/04	29/04	17	0.5	17
2018	19	3130	310	11	12/04	29/04	17	30	31/03	23/04	23	0.4	29
	23	3260	670	23	06/04	18/04	12	28	21/04	06/05	15	0.8	30
	182	6840	220	8	12/04	25/04	13	69	01/04	24/04	23	0.1	24
Mean ± S.E.		3776 ± 650	330 ± 71	13 ± 3			15 ± 4	44 ± 10			22 ± 2	0.4 ± 0.1	27 ± 2

Observations were carried out during two different years, and nests were observed during most days during the period that reproductive individuals emerged from nests. Worker and queen cells were quantified once the reproductive flight period was over.
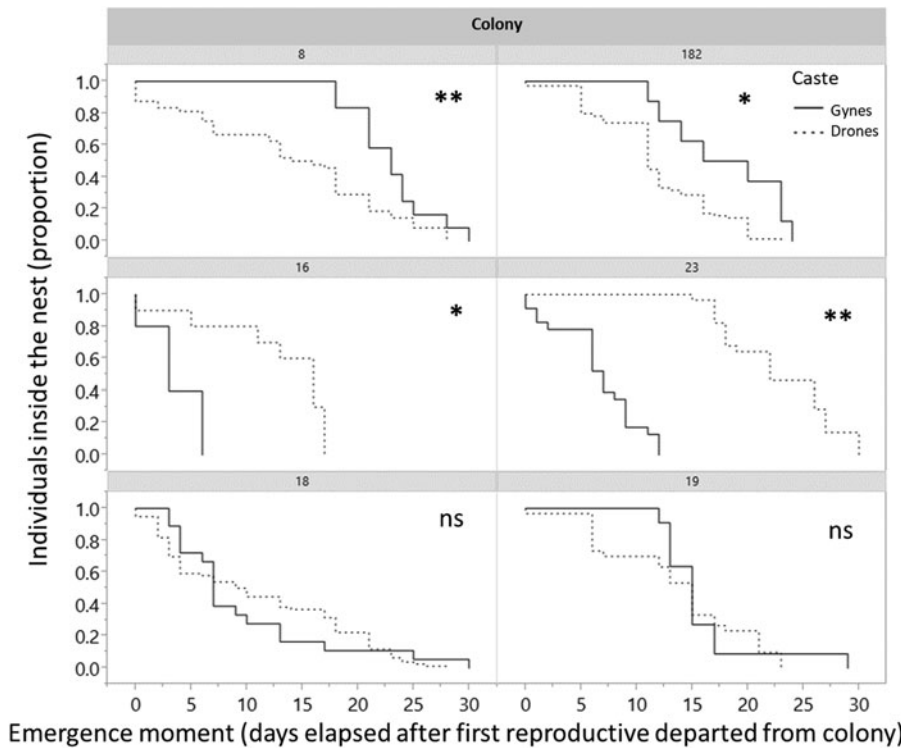
Figure 1. Nest departure of reproductive individuals in six observation *Vespula germanica* colonies during 2017 and 2018. Colonies were observed throughout the reproductive flight season and emerging gynes and drones were registered for 10 min every hour on most days during the reproductive period. Two colonies had a significant departure of drones ahead of gynes, while in other two colonies (8 and 182), gynes significantly left before drones (16 and 23) and in two colonies we observed no significant differences in the departure of reproductive castes (18 and 19). *$P < 0.05$, **$P \leq 0.01$ and ns: $P > 0.05$.

$P = 0.02$, d.f. = 1, risk = 2.1 and $\chi^2 = 6.8$, $P = 0.01$, d.f. = 1, risk = 2.63, respectively). In pattern 3, gynes and drones emerged concurrently with no statistical temporal difference (nests 18 and 19, $\chi^2 = 0.00003$, $P = 0.99$, d.f. = 1, risk = 1 and $\chi^2 = 0.1$, $P = 0.8$, d.f. = 1, risk = 1.1, respectively).

For each colony, castes were re-assigned a *post-hoc* categorical variable with three levels contemplating the order of within-nest departure pattern regardless the caste (i.e., early vs. opposite caste ('early'), late vs. opposite caste ('late') or no difference between castes ('same')). Overall, statistical differences were registered between the three levels ($\chi^2 = 45.75$, $P < 0.0001$, d.f. = 2). *Post-hoc* comparisons between levels indicate statistical differences between those castes leaving the nest early vs. late ($\chi^2 = 42.4$, $P < 0.0001$, d.f. = 1, risk = 2.8) and 'same' vs. late ($\chi^2 = 32.5$, $P < 0.0001$, d.f. = 1, risk = 2.5), while castes emerging early did not differ with 'same' ($\chi^2 = 0.81$, $P = 0.36$, d.f. = 1). To convey an idea of the magnitude of these differences in nest-departure behaviour, 50% of the reproductives that emerged early, did so within the first 10 days of the departure period of the nest, while 50% of the caste that lagged behind, took more than double the time to leave the nest, doing so by day 20 (fig. 2).

Daily departure behaviour

Analysis of daily departure patterns indicates that all reproductive individuals emerged between 10 and 16 h. Overall results indicate that gynes left the nest between 11 and 16 h (mean ± standard error: 13 ± 1 h, $n = 77$) while drones did so between 10 and 16 h (mean ± standard error: 12 ± 1 h, $n = 261$), with significant differences between the two sexes (Cox Proportional Hazards $\chi^2 = 20.2$, $P < 0.0001$, d.f. = 1). Using the absolute hour as the response variable, significant differences were found through additional Cox Proportional Hazards models between different years ($\chi^2 = 35$, $P < 0.0001$, d.f. = 1) and nests ($\chi^2 = 38.5$, $P = < 0.0001$, d.f. = 5),

therefore further analyses were carried out using a normalized departure time, expressed for each individual, as the number of hours elapsed after the first individual left that day within each nest. Using this normalized response variable, we found no statistical differences between years ($\chi^2 = 0.02$, $P = 0.9$, d.f. = 1) or nests ($\chi^2 = 8.55$, $P = 0.1$, d.f. = 5), and found differences between castes ($\chi^2 = 11.5$, $P = 0.0007$, d.f. = 1), with drones leaving 1 h ahead of gynes (risk = 1.5, n gynes = 77, n drones = 261) (fig. 3). Furthermore, this difference was driven by those gynes leaving on the same days as drones, delaying nest departure by 1.5 h ($\chi^2 = 7.38$, $P = 0.007$, d.f. = 1, n gynes = 44, n drones = 118). The time gynes and drones left on days without the opposite caste was not significantly different ($\chi^2 = 0.3$, $P = 0.57$, d.f. = 1, n gynes = 33, n drones = 143) (fig. 4).

The ambient temperature at the moment of departure from nests was statistically different between gynes and drones, with gynes emerging at higher temperatures than drones (mean gynes = $14. \pm 0.3$°C, $n = 77$, mean drones = 13 ± 0.2°C, $n = 261$; Wilcoxon $\chi^2 = 19.5$, $P < 0.0001$, d.f. = 1) (fig. 5).

Discussion

To our knowledge, this is the first study to report nest-departure behaviour of *V. germanica* gynes and drones with an hourly resolution. A number of studies in the past looked at the within-nest phenology of social vespids, in particular, the moment of emergence from the pupal state of different castes and established that drone adult departure in *V. germanica* and other species such as *V. vulgaris* and *V. maculifrons*, occurs several weeks ahead of gynes (Thomas, 1960; MacDonald and Matthews, 1981; Fordham *et al.*, 1991; Leathwick *et al.*, 1999). These studies were carried out by counting individuals from excavated nests throughout the year, with the inherent limitation that the actual timing of nest departure could not be determined. First, our
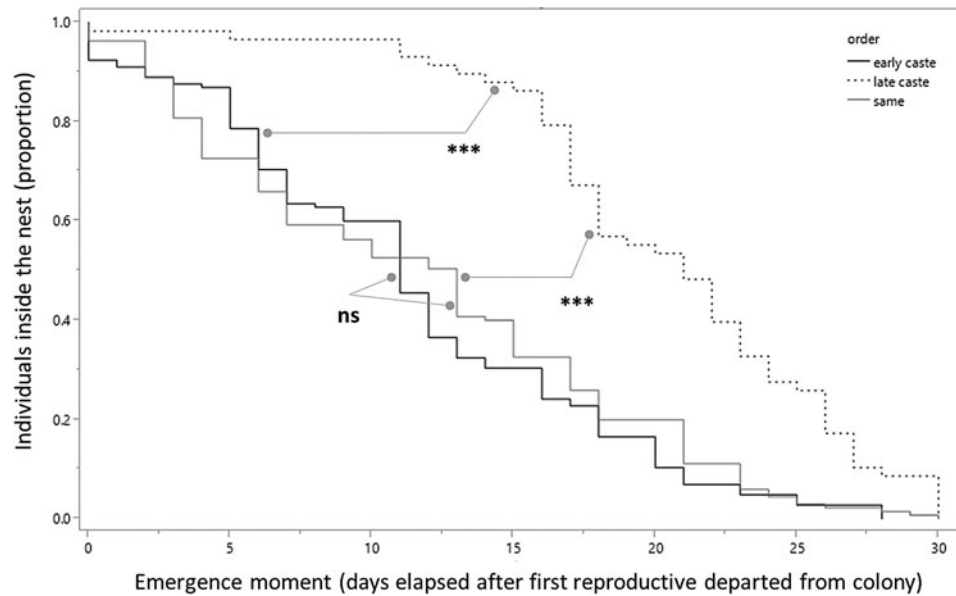
Figure 2. Within-colony departure patterns of reproductive castes in *Vespula germanica*. The traffic of six colonies was registered during the reproductive period. In most colonies (four out of six), a significant difference was observed of one of the castes emerging ahead of siblings of the other caste: early vs. late ($\chi^2 = 42.4$, $P < 0.0001$, d.f. = 1, risk = 2.8), same vs. late ($\chi^2 = 32.5$, $P < 0.0001$, d.f. = 1, risk = 2.5), while castes emerging early did not differ with same ($\chi^2 = 0.81$, $P = 0.36$, d.f. = 1). ***: $P \leq 0.001$ and ns: $P > 0.05$.
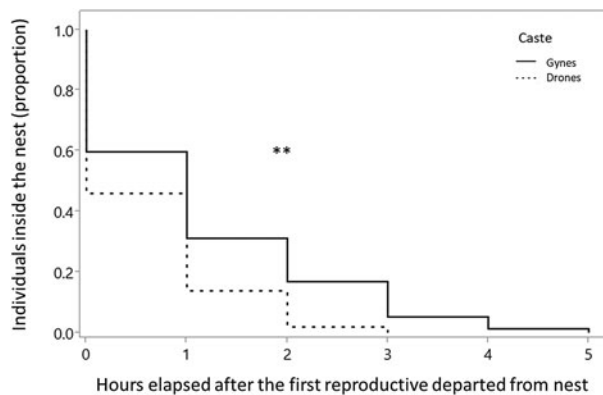

Figure 3. Daily nest departure of *Vespula germanica* gynes and drones. The response variable was normalized for every individual considering the difference in hours vs. the first reproductive that left the nest that day. During the day, drones tended to leave the nest ahead of gynes (Cox Proportional Hazards $\chi^2 = 8.28$, $P < = 0.004$, d.f. = 1, risk drones leaving before gynes = 1.46, n gynes = 77, n drones = 261).

study shows that *V. germanica* sexual individuals leave the nests permanently, with neither gynes nor drones returning to the parental nests. Second, and despite the fact that we did not detect a tendency for one particular sex to leave the nest before the other, we did note asynchronic nest departure between gynes and drones in some colonies, with this asynchronous departure not fixed to a particular caste leaving before the other one, as gynes or drones delayed their departure as a function of the departure of the opposite sex, depending on the colony. At a higher temporal resolution (i.e., within the day), drones consistently began to leave nests 1 h before gynes and this difference was driven by those individuals that left on the same day as did the opposite-sex kin.

Generally, reducing the risks of inbreeding can be achieved through different direct and indirect mechanisms such as the recognition of familiar cuticular hydrocarbons, multiple mating and variations in the dispersal capabilities of opposite sexes (Strassmann, 2001; Beani *et al.*, 2019). In *V. germanica*, some mechanisms that could contribute to this have been reported in the past: queens are typically polyandrous, mating with up to seven drones (Goodisman *et al.*, 2002), a reproductive trait believed to contribute to the reduction of inbreeding risk (Tregenza and Wedell, 2002; Cornell and Tregenza, 2007). Additionally, past studies have determined in laboratory bioassays that gynes and drones have differential flight potential, with drones flying considerably less than gynes, suggest that this could contribute to the spatial segregation of the sexes (Masciocchi *et al.*, 2016). Interestingly, Goodisman *et al.* (2002) suggest that gynes appear not to discern kin and non-kin, as evidenced indirectly by polymorphic microsatellite markers used to study patterns of reproduction, indicating that mating occurs at random (i.e., mating can occur among kin). If effectively females do not avoid drone kin as mating partners, additional and complementary mechanisms such as an asynchronous nest departure behaviour could effectively contribute to reducing inbreeding in *V. germanica*.

Our results indicate that reproductive castes leave the nest permanently as neither gynes nor drones returned to the nest. It is important to note that we observed departures during 10 minutes for every hour and it is possible that gynes and drones returned to colonies between observation bouts, but this is unlikely. We also found no reproductives inside the nests once the departure period finished. This is the first study to show unequivocally that *V. germanica* reproductives do not return to the nests after departure. Even though there have been suggestions that mated gynes could return to nests in *V. germanica* (e.g., Thomas, 1960), we did not observe such behaviour in the duration of our study. Other social hymenopteran reproductives do effectively return to the nests after copulation, for instance, *Apis mellifera* drones can return up to several times to the nest after mating flights
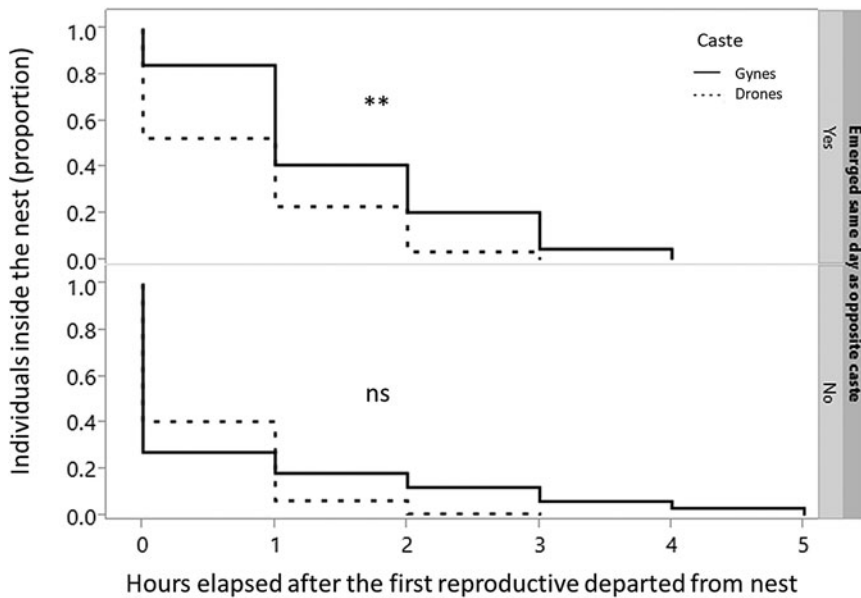
Figure 4. Daily departure from parental nests of gynes and drones on days with and without the opposite caste emerging. The response variable was normalized for every individual considering the difference in hours vs. the first reproductive that left the nest that day. When emerging on same days as kin-drones, gynes significantly delayed the hour of departure (Cox Proportional Hazards $\chi^2 = 7.38$, $P = 0.007$, d.f. = 1, n gynes = 44, n drones = 118), while when emerging on separate days, this moment of nest departure was not statistically different (Cox Proportional Hazards $\chi^2 = 0.3$, $P = 0.57$, d.f. = 1, n gynes = 33, n drones n = 143).
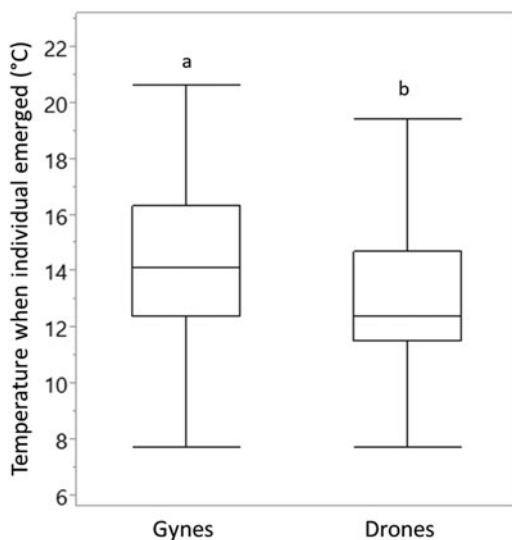

Figure 5. Temperature at the moment reproductive *Vespula germanica* individuals left their parental nests during days with or without kin of the opposite sex. Gynes emerged at a higher temperature than drones (mean gynes = 14.4 ± 0.3°C, $n = 77$, mean drones = 13 ± 0.2°C, $n = 261$; Wilcoxon $\chi^2 = 19.5$, $P < 0.0001$, d.f. = 1). The top and bottom boundaries of the boxes indicate 50% of the data spread. The line within the boxes marks the median value. Whiskers indicate the range of the data. Different letters denote significant differences (Wilcoxon signed-rank test $P < 0.05$).

(Koeniger *et al.*, 2005). Conversely, in the bees of the genus, *Melipona* (Apidae, Meliponinae) drones do not return to the nests, offering the advantage of conserving energy by not having to fly back to the colony (Van Veen *et al.*, 1997). In the case of *V. germanica*, spending the nights away from the nest would imply a higher risk of predation and exposure to adverse climatic conditions, but could represent the advantage of comparatively higher dispersal rates than those species that do return to the nest, in addition to alleviating the nest from feeding the males.

In contrast to previous accounts of protandry in *V. germanica*, where drones were observed in the field before females (Spradbery, 1973), our study suggests a high between-nest

variability with no fixed order in departure patterns for gynes and drones. In four out of six nests, we observed that the departure of one of the sexes was significantly delayed vs. the other, as evidenced by the early departure of ca. 50% of reproductives of the 'early caste' by the time only 10% of the late caste did so. Conversely, in the other two observation colonies, reproductive nest-departure had no detectable temporal segregation between siblings of the opposite caste at the seasonal scale. The variability observed in our study, where different colonies behave differently, could present advantages from several points of view. On the one hand, having such between-nest variability could spread the risk in relation to environmental uncertainty (Hopper, 2002) especially considering that the mating period in *V. germanica* occurs in autumn, when night temperatures can fall below 0°C. Additionally, an asynchronic departure pattern could foster the coexistence of opposite sex non-kin, thus increasing the probabilities for early drones to coexist temporally with early non-kin gynes, during what is generally a short reproductive season.

Within-day departure patterns between gynes and drones indicate that males tend to leave the nest earlier than gynes, with a mean difference of ca. 1 h. Even though such a small difference in departure timing would probably contribute to a lesser degree than emerging on different days, it could still be important at reducing inbreeding risks, especially when considering that the time difference became larger when opposite-sex kin emerged on the same day. In the solitary parasitoid, *Cotesia glomerata* males emerge a few hours ahead of their sisters, thus separating them temporally (Mazzi *et al.*, 2011). It is possible that different thresholds in temperature could drive the observed departure timing differences between sexes, since earlier in the morning when drones begin to emerge, temperatures are lower.

The observed departure from nests, where some colonies and individuals leave together with the opposite caste while in other nests temporal segregation seems to occur and is not fixed to a particular caste, could have important implications in the invasion process by balancing inbred and outbred matings. For instance, such flexible behaviour would favour inbred matings at the early stages of the invasion process or the leading edge of range expansion, when the probability of finding genetically-

distant partners is low. Variable reproductive behaviour at the colony level has been observed by Bogo *et al.* (2018) who investigated the inbreeding risk in colonies of *Bombus terretris*, and observed a strong effect on colony developmental characteristics on the reproductive strategies that in turn affected the risks toward inbreeding. Variable behaviour between colonies has also been recorded in other aspects of colony life, such as foraging and defence tasks, with studies showing variable colony behaviour in the black harvester ant, *Messor andrei* (Hymenoptera: Formicidae), where responsiveness to food baits and disturbance varies between colonies according to nest structure and weather (Pinter-Wollman *et al.*, 2012). Plasticity in social hymenopterans is not uncommon and has been suggested as one of the key drivers for their success as invasive species (Manfredini *et al.*, 2019).

Future studies of the reproductive biology of *V. germanica* and other social Vespids, including genetic studies within and among nearby colonies, should help establish any particular factors in the life history of the colony, such as size, diet or interactions with the environment, contribute to the observed nest departure patterns. This would help establish how the species overcomes the aforementioned disadvantages of reduced genetic diversity while achieving local dominance and territorial expansion could help explain mating patterns (Schrieber and Lachmuth, 2017). The present study extends our current understanding of the reproductive biology of the invasive social wasp *V. germanica*. Obtaining a deep understanding of the reproductive biology of invasive Vespidae in general and *V. germanica* in particular could help understand the drivers for their success as an invasive species and open opportunities for alternative management tools.

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