

Foraging Behaviour
and
Individuality
in the
Common Wasp
(*Vespula vulgaris*)

by

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Abstract

The extreme ecological success of insect societies is frequently attributed to the division of labour within their colonies (Chittka & Muller, 2009; Holldobler & Wilson, 2009; E. Wilson & Hölldobler, 2005). Yet, we are far from understanding the causes and consequences of division of labour, implying workers' specialization (Chittka & Muller, 2009; Dornhaus, 2008). Moreover, little studied is the behaviour of individual workers (Jeanson & Weidenmüller, 2013). Social wasps (Hymenoptera: Vespidae) have received less attention than social bees and ants, and our knowledge of basic aspect of their ecology is still poor (Jeanne, 1991; Greene, 1991). With my thesis, I aimed to contribute to a better understanding of the common wasp (*Vespula vulgaris*) foraging ecology and organization of labour. With a particular attention to their foraging behaviour, I investigated the inter-individual variability among wasp workers and their cooperation.

My thesis shows evidence of information sharing and co-ordination in *V. vulgaris* foragers' activity. In fact, the discovery and choice of resources by wasp foragers was assisted by information provided by experienced nestmates (Chapter 2). When resources known to portion of the workforce became newly available, the foraging effort of the whole colony increased. My observations of common wasps are hence consistent with foraging activation mechanisms and suggest piloting (in which one individual leads one or more nestmates to a resource) as a possible foraging recruitment mechanism in social wasps.

I found huge variation in lifetime activity, task performance, and survival among common wasp workers (Chapter 3). Some individuals specialized on alternative foraging tasks over their lifetime, and a minority individuals performed a disproportionately high number of foraging trips (elitism). Foragers appeared to become more successful with age, accomplishing more trips and carrying heavier fluid loads. Compared to smaller nestmates, larger wasps contributed more to the colony foraging economies. High mortality was associated with the beginning of the foraging activity, relative to lower mortality in more experienced workers.

I evaluated the performance of common wasp workers within the same insect colony, and found empirical support for the hypothesis that specialist foragers are more efficient than generalists (Chapter 4). In fact, *V. vulgaris* behavioural specialists performed more trips per foraging day and their trips tended to be shorter. Despite their more intense foraging effort, specialists lived longer than generalists.

I investigated the intra-colonial variation in the sting extension response (SER) of common wasps, measured as a proxy for individual aggressiveness (Chapter 5). I found that wasps vary greatly in their SER and that individuals change during their life. Aggressive individuals tended to become more docile, while docile individuals more aggressive. Older wasps tended to be more aggressive. Wasp size was not significantly related to the SER. Wasp foragers had a less pronounced sting extension than individuals previously involved in nest defence. For the same individual, the aggressive response was proportional to the intensity of the negative stimulus.



Acknowledgements

This dissertation is based on my own independent work, carried out at Victoria University of Wellington between 2012 and 2015, but it would have been impossible without the help I received.

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Dulcis in fundo, thanks to my family. Grazie, Antonio and Pina, for raising me and Elio. Arigatou, Akari, for being always by my side, and for our daughters, Luna and Asia. We made it!



Statement of Authorship

I, Davide Santoro (DS), hereby declare that this thesis is my own work and that all sources quoted, paraphrased or otherwise referred to have been properly acknowledged in the references at the end of each respective chapter. To the best of my knowledge, this thesis neither contains material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institutes of higher learning, except where due to acknowledgement it has been made clear in the text.

Chapters 2, 3, 4, and 5 of this thesis were written as independent manuscripts intended for publication, and were written in first person plural tense. In all cases, the manuscripts were written by DS. For all the chapters, Professor Philip J. Lester (PJL) financed the fieldwork and provided advice throughout the project, contributing to the editing of all the thesis chapters. Dr. Stephen Hartley (SH) provided materials, advice and contributed to the editing of all the manuscripts. Dr David Maxwell Suckling (DMS) provided advice and contributed to the editing of Chapters 2 and 5, hosting me in the Plant & Food Research Ltd laboratory in Lincoln and providing logistic support in 2012 and 2014.

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Chapters 3 and 4 were written for intended publication in *Behavioural Ecology and Sociobiology* and *Proceedings of the Royal Society B* respectively, but they are yet to be submitted. Author contributions: DS conceived, designed the study and conducted the fieldwork. PJL provided advice during the experimental design, on the statistical analyses and helped draft the manuscript. SH provided advice on the statistical analyses and helped draft the manuscript.

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Foraging Behaviour and Individuality in the Common Wasp (*Vespula vulgaris*)





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Chapter 1

General Introduction

Insect societies show ecological dominance, extreme diversity, and almost cosmopolitan distribution, achieving the highest levels of social organization and complexity (eusociality) (Wilson 1971; Michener 2000; Holldobler and Wilson 2009). Eusocial insects are characterized by overlapping adult generations, cooperative brood care, and reproductive division of labour (Wilson 1971; Wilson and Hölldobler 2005). Division of labour, the task differentiation among colony members, is hence one mainstay of eusociality, and is considered to be a major cause of the ecological success of the insect societies (Wilson 1975; Chittka and Muller 2009). Aside from the fundamental divergence between reproductive and worker caste, individuals vary within one caste (Jeanne 1991). In fact, within one insect colony, the workforce is organized into groups performing different tasks (division of labour or task allocation), and each task can require the coordination of workers performing different activities (task partitioning) (Anderson and Ratnieks 1999).

Two general patterns of division of labour are recognized in social insects: temporal and morphological polyethism (Beshers and Fewell 2001). Temporal (or age) polyethism refers to age-correlated patterns of task performance. Temporal polyethism is widespread in social insects and typically follows the pattern of young workers performing tasks within the nest and older workers performing outside, more dangerous tasks such as foraging and colony defence (Robinson 1992). Morphological polyethism occurs when workers' size and/or shape is related to the task performed. Morphological polyethism has been mostly investigated in those social insect species (termites and ants) with distinguishable subcastes within the worker caste, where the most common specializations are for defence and foraging (Beshers and Fewell 2001).

Differences among individuals from the same insect colony go beyond what can be explained by polyethic models (Jeanson and Weidenmüller 2013). Although data available are still limited to a few species, and mostly cover relatively short time periods over insects' lives, there is some evidence suggesting that there are enormous differences in individual activity within the colonies of

bees, wasps and ants colonies (O'Donnell and Jeanne 1992a; Hurd et al. 2003; Polidori et al. 2006; Tenczar et al. 2014; Perry et al. 2015). For some colonial species, a minority of insect workers, often referred to as “elite”, seem to be consistently hyper-active and productive (Hurd et al. 2003; Tenczar et al. 2014; Charbonneau and Dornhaus 2015). On the other hand, the vast majority of workers spend most of their time inactive, and in ants, one out of four workers can look completely inactive, so that “laziness” seems to be the rule (Charbonneau and Dornhaus 2015).

It appears that insect workers from the same colony can greatly differ in frequency, rate, sequence and ontogenetic timing of task performance, but surprisingly few studies have deepened our understanding of insect workforce organization focusing on the variation at the individual level (Jeanne 1991). Although a key feature of division of labour is worker specialization (Beshers and Fewell 2001), we are still far from understanding the adaptive benefits of this phenomenon (Dornhaus 2008; Chittka and Muller 2009).

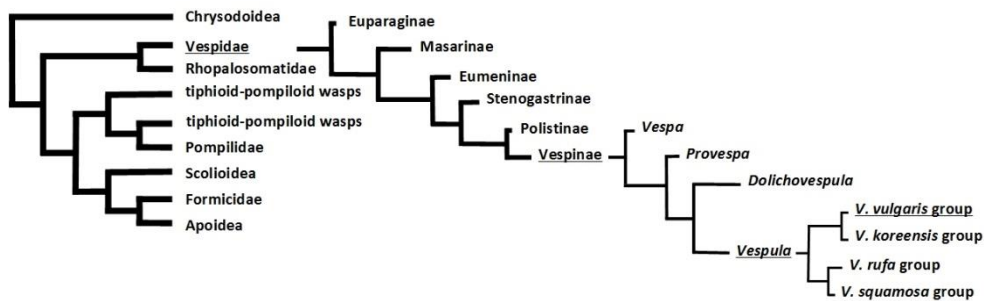


Fig. 1.1 Relationships among the Aculeate Hymenoptera resolved by next generation sequencing (redrawn from Johnson et al. 2013) and morphological analysis of the subfamilies of Vespidae and genera of Vespinae, showing also the interrelationships of the species groups in *Vespula* (redrawn from Carpenter, 1991). Phylogenetic relationships within Vespinae are not yet clearly resolved, but morphological and molecular studies concord on the monophyly of the *Vespula vulgaris* species group and the sister relationship between the *V. rufa* and *V. squamosa* groups (Loope et al. 2014; López-Osorio et al. 2014)

1.1 Wasps, yellowjackets and *Vespula*

The Vespidae are a large (nearly 5000 species), diverse, and cosmopolitan family of wasps, including nearly all the known eusocial wasps (Pickett and Wenzel 2004). Within the family Vespidae, the subfamily Vespinae includes hornets (*Vespa* and *Provespa*) and yellowjackets (*Dolichovespula*, and *Vespula*) (Fig. 1.1). The vespine group encompasses about 60 described species, originally distributed throughout the Holoarctic Region and the Oriental tropics (Greene 1991). The 40-48 yellowjacket species currently recognized (Lopez-Osorio et al. 2014) are widely distributed in the Northern Hemisphere, but they have been inadvertently introduced by humans in many non-native areas, also in the Southern Hemisphere (Beggs et al. 2011). These social wasps have invaded countries such as Hawaii, Argentina, South Africa, Australia, Tasmania and New Zealand, and caused major ecological impacts in the invaded range (Beggs 2001; Lester et al. 2014)

Yellowjackets show very little morphological divergence between species (Archer 1989; Archer 2008) (Fig. 1.2), and are almost indistinguishable if colour patterns and characters of male genitalia are ignored (Yamane et al. 1980; Akre et al. 1981). Moreover, aside from two departures from the norm (perennial colonies and social parasitism, obligate or facultative), the life history within this group of social wasps is extremely consistent (Greene 1991). Despite these common features, yellowjackets show a very remarkable behavioural divergence (Greene 1991; Lopez-Osorio et al. 2014). *Dolichovespula* and *Vespula* species exhibit far more diversity in their social biology than any other group of annual eusocial insect, showing mature colonies counting from a few dozen to hundreds of thousands individuals (Greene 1991). Mainly on the basis of colony size, yellowjackets are traditionally divided in two fundamentally different groups: the small colony yellowjackets (*Dolichovespula* and *V. rufa* species group) and the large-colony yellowjackets (*V. vulgaris*, *V. koreensis* (?), and *V. squamosa* species groups) (Akre et al., 1981; Archer, 2008; Greene, 1991) (Fig. 1.1).

Half a century ago, the PhD dissertation of N. B. Potter (1964) shed a light on many aspects of the common wasp (*V. vulgaris*) biology. Potter's work has represented a milestone in our understanding of the biology of yellowjacket wasps, particularly in relation to their foraging behaviour, and has been directly or indirectly reported in all the major reviews of the behavioural ecology of social wasps published since then (Archer, 2012; Edwards, 1980; Matsuura & Yamane, 1990; Ross & Matthews, 1991; Spradbery, 1973). In the last fifty years, a growing body of literature has focussed on the social biology of other yellowjacket species, particularly during the 1970s and 80s, and has revealed a surprising inter-specific diversity in social complexity and behavioural ecology, when considering the limited number of species and their close phylogenetic relationships (Greene 1991).

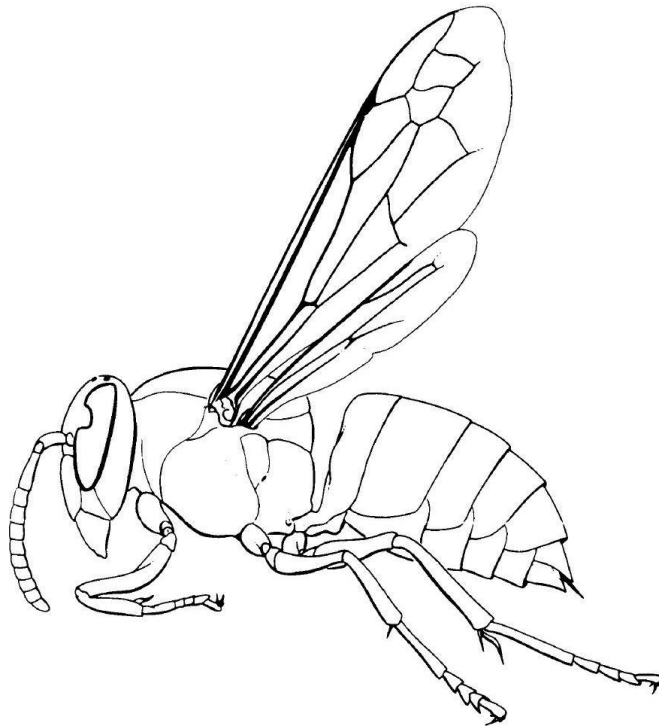


Fig. 1.2 Yellowjacket wasp (*Vespa*, *Dolichovespula* spp.) body structure. Adult worker (Akre et al. 1981).

1.2 *Vespula vulgaris* life history

Similar to other yellowjackets, common wasp (*V. vulgaris*) colonies are normally characterized by an annual life cycle and the alternation of solitary and social phases (Fig. 1.3). By mid-winter, *V. vulgaris* is typically represented only by hibernating, fertilised queens (Edwards 1980). Hibernation takes place in dry cavities (for example, under tree bark). During late spring or early summer, the queens start to wake up and, after two or three weeks of feeding, each one begins to search for a suitable site to construct a nest. Intra- and inter-specific fights between wasp queens and nest usurpation is common at this point (Greene 1991). Nests can be started and grow both in aerial and subterranean sites (Spradbery 1973).

In rural areas, *V. vulgaris* is usually a subterranean nester, often nesting in old rodent burrows. In urban areas, covered aerial sites are more frequent, sometimes even more frequent than subterranean sites. Aerial sites include sheds, cavity walls, roof spaces, beehives, dense vegetation, tree hollows and rotten tree stumps (Spradbery 1973). Common wasps' nests are found in both lowland and mountainous regions above 1000 m (Clapperton et al. 1994). Nests are made of wood pulp obtained by mixing scraped and chewed wooden fibres with saliva. *Vespula vulgaris* nests are characterized by mottled light brown envelopes, differing from congeneric and often coexisting species such as *V. germanica*, whose nests are grey (Leathwick, 1997).

Once she has chosen the nesting site, the founding queen builds a vertical stalk with the bases of several cells at its lower end, plus part of the surrounding envelope. Cells are hexagonal and hang vertically, forming one horizontal comb (Akre et al. 1981). One egg is laid in each cell as soon as it has been completed. About one month after the beginning of oviposition by the queen, the first workers emerge. When the first workers start provisioning the nest, the queen stops foraging and devotes her life to oviposition (Matsuura & Yamane, 1990). As worker numbers increase, the queen-built comb, surrounded by a lantern-shaped

envelope, is enlarged. Cells are added to the periphery of the comb. Throughout the season, as in other large-colony yellowjacket species, new combs are subsequently suspended from older ones and expanded (Spradbery 1973). The average size of the cells increases, as it does the average worker size (Spradbery 1972). The envelope is widened and thickened. In subterranean nest, soil is mined and removed from the ground to enlarge the cavity and accommodate the expanding nest. When the worker-raised workers start emerging, the colony grows at an increasing rate (Spradbery 1973).

By the end of the summer, male (drone) production begins. Drones develop from unfertilized eggs. The drone-worker ratio increases and drones can outnumber workers. In the meanwhile, small cell construction stops and larger cells dedicated to the production of queens are built. Nest expansion reaches at this point its climax (Archer 2008). Although there is a lot of variation among colonies, one *V. vulgaris* nest at this stage can be composed on average of nine combs, 6000 to 13000 worker cells, about 3000 workers, and thousands of larvae and pupae (Potter 1964; Archer 2008). The virgin queens start emerging at the beginning of the autumn and spend two to six weeks in their natal nest, occasionally foraging outside. Mating follows, seeming to occur both inside and outside the nests. Queens mate with several drones (polyandry), and males are able to mate more than once (Strassmann 2001; Loope et al. 2014). Mated new queens subsequently look for a shelter to hibernate. After the bulk of reproductive individuals have left the nest, the autumnal weather deterioration leads to a worsening food shortage inside the nest. Cannibalism rises, the old queen dies, and normally the colony collapses completely with the arrival of the winter (Spradbery 1973).

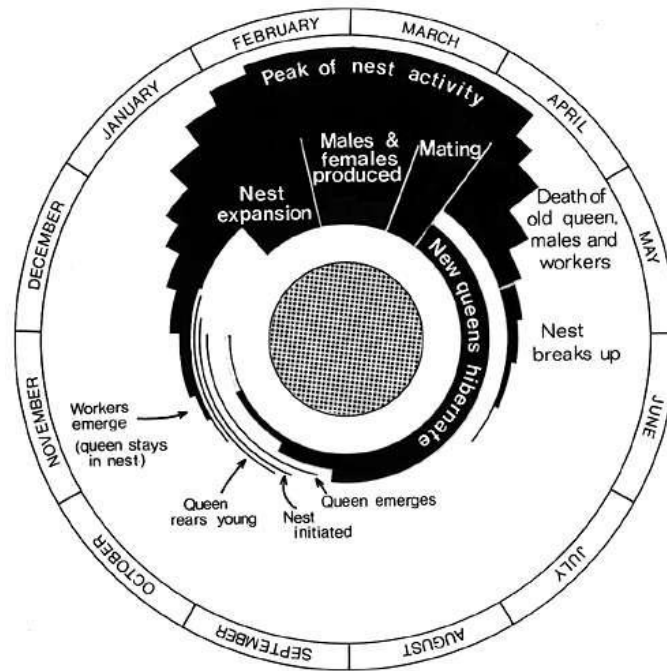


Fig. 1.3 *Vespa vulgaris* life cycle in New Zealand. Landcare Research © (modified from Edwards 1980).

1.3 Polyethism in wasps

Similar to eusocial bees and ants, there is some evidence that social wasp workers show temporal polyethism, changing tasks in relation to their age (Potter 1964; Akre et al. 1976; Hurd et al. 2007; Kim et al. 2012). Within the Vespinae, the occurrence of temporal polyethism seems to be variable, with *Vespa* and *Dolichovespula* showing no or weak tendency, and *Vespula* showing clearer ontogenetic transitions (Fig. 1.4). Yet, polyethism in vespulids does not seem to be as marked as, for example, in honey bees, and a lot of inter-individual variability and plasticity has been mostly anecdotally reported (Spradbery 1973; Edwards 1980; Hurd et al. 2007). In honey bees, *Apis mellifera*, domestic duties such as nursing or ventilating are performed during the first days of life, with guarding and foraging performed later (Seeley 1982). Vespulids do not seem to switch as abruptly from one task to another during their life, and perform multiple tasks on short time-scales (Hurd et al. 2003; Hurd et al. 2007).

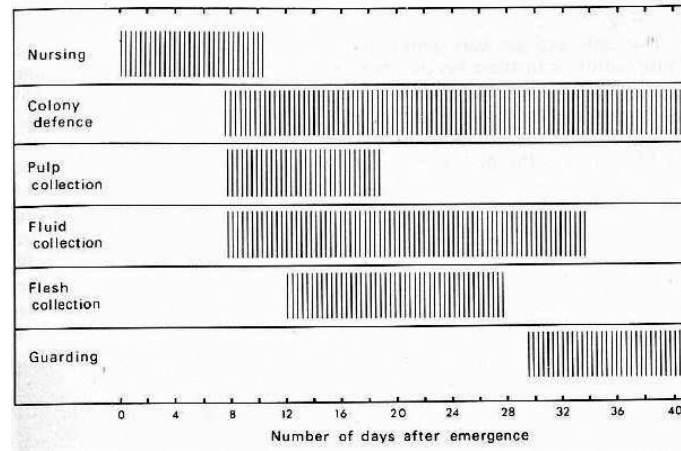


Fig. 1.4 Temporal polyethism in *Vespula vulgaris* workers, after Potter 1964 (Edwards 1980).

Polyethic transitions in *Vespula* wasps have been studied within foraging activity, and despite very small sample sizes for species such as *V. vulgaris* (Fig. 1.5), common patterns were found among species. During the first foraging days, the dominant activity is wood-pulp collection. Flesh collection tends to occupy the middle period of adult life. Throughout the foraging career, there is a progressive increase in fluid collection (Brian and Brian 1952; Potter 1964; Hurd et al. 2007; Kim et al. 2012). The number of foraging trips performed by common wasp individuals appears to mostly decrease with their age (Fig. 1.6). Among the other workers' activities, aggression of nestmates (mauling), nest defence and guarding were observed only in individuals older than eight days in *V. pennsylvanica*, *V. atropilosa* (Akre et al. 1976), and *V. vulgaris* (Potter 1964) (Fig. 1.4).

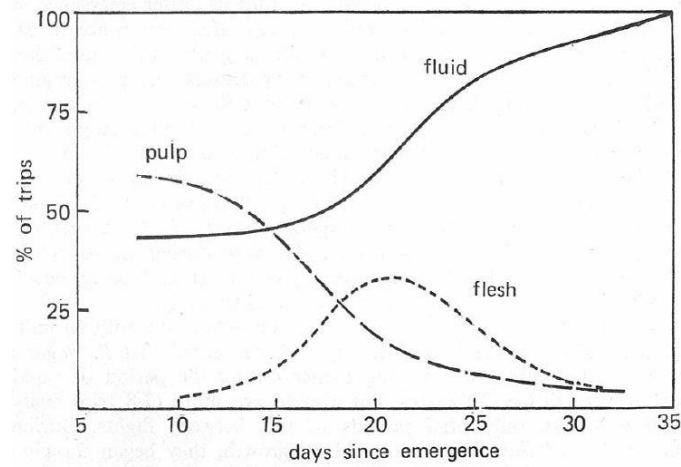


Fig. 1.5 Ontogenetic changes in foraging activity of 30 *Vespula vulgaris* workers, followed for a variable number of days. After Potter 1964 (Spradbery 1973)

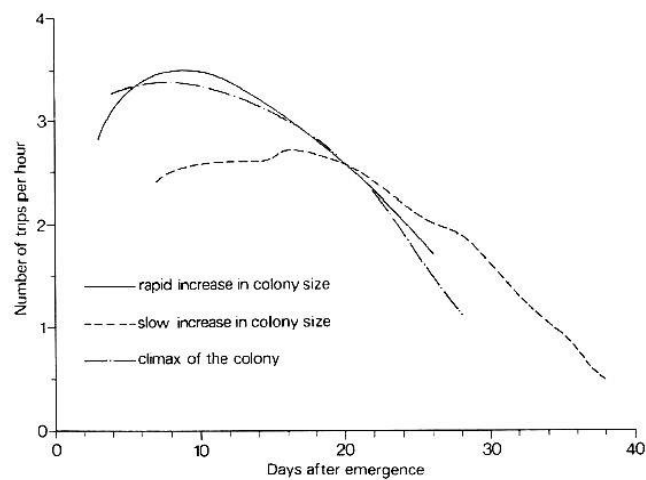


Fig. 1.6 Variation in number of foraging trips with individual age and stage of colony development *Vespula vulgaris* workers, after Potter 1964 (Edwards 1980).

1.4 Social wasps and *Vespula* foraging

Social wasps forage for water, pulp, carbohydrates, and animal protein. For wasp foragers, individual and opportunistic exploitation of cues (passive sources of information, aspects of the physical or social environment that may convey information incidentally) seems to be the rule (Raveret-Richter 2000). Social wasps are generalist foragers that use a variety of mechanisms to locate and choose resources. Wasps are influenced by past foraging experience and

have the ability to learn landmarks through orientation flights and to promptly associate colours and odours with food rewards (Raveret-Richter 2000). Foraging wasps appear to utilize whatever combination of cues provides the greatest amount of information (Jeanne and Taylor, 2009). Apparently, visual cues determine the area in which they search intensively for prey, while landing responses are elicited mainly by odour cues (Moreyra et al. 2006). On the basis of their learning abilities, individuals return to foraging sites where they have been successful and may feed repeatedly on the same kind of resource, thus acting as facultative specialists (Raveret-Richter, 2000). This characteristic feeding-site fidelity can persist also after the food source is depleted (Spradbery 1973). The frequency with which an individual wasp returns to a site seems to depend on its previous rewards there (Free, 1970) and memory extinction is related to the number of visits previously performed (Lozada and D’Adamo 2006). Although empirical evidence is weak, alternative foraging tactics have been reported for different wasp foragers. Some individuals show site fidelity, while others are not constant to one location and appear to search in widely separated areas (Akre et al. 1975). Once the food has been discovered, wasp foragers can show aggressive behaviour (directed towards conspecifics or heterospecifics), aimed to the monopolization of the resource (Spradbery 1973; Grangier and Lester 2011).

Social wasp species within one genus, such as *Vespula*, differ in their foraging habits and resource exploitation ability (Raveret-Richter 2000, Jeanne and Taylor 2009, Leathwick 1997, Harris and Oliver 1993, Harris et al. 1994). Large-colony yellowjackets of the *V. vulgaris* species group - including the common wasp (*V. vulgaris*) and the German wasp (*V. germanica*) - are paradigms of extreme foraging generalism. These species are characterized by very peculiar scavenging and necrophagic habits, setting them apart from other *Vespula* and social wasp species, and show among the most eclectic diets known for insects (Greene 1991). Aside from preying on virtually any invertebrate they can overcome, they also take flesh from struggling animals such as injured barnacles (Yamane et al.

1980) or stranded fishes (Fig. 1.7), imbibe blood from vertebrates after producing wounds, scavenge on vertebrate and invertebrate carrions, feed on fruits as well as other carbohydrate sources such as honeydew, and consume a wide array of processed human food, both carbohydrate and proteinaceous (Spradbery 1973).

Within the *V. vulgaris* species group, common and German wasps exhibit alternative provisioning tactics in different environmental conditions. *Vespula vulgaris* hunt slowly and deliberately around stems, in bushes and under leaves low down near the ground, whereas *V. germanica* hunt in open for prey, flying swiftly among vegetation until prey is encountered, then rapidly pouncing on it (Jeanne and Taylor 2009). In the New Zealand beech forests, common wasps have been observed foraging for protein sources mainly on shrubs and trees, while in contrast German wasps forage primarily amongst the forest litter (Harris 1991). In these forests, *V. vulgaris* collected more Hemiptera and Lepidoptera, *V. germanica* more Orthoptera and Hymenoptera (Harris 1991). In scrubland-pasture, *V. vulgaris* collected mainly Lepidoptera; *V. germanica* mainly Diptera (Harris and Oliver 1994). These species specific prey spectra possibly mirrored differential microhabitat use patterns. In the beech forests common wasps were also found to be more efficient in harvesting the honeydew produced by the beech scale insect *Ultracoelostoma assimile* (Harris et al. 1994). In this ecosystem, *V. vulgaris* has displaced *V. germanica* in a few years (Thomas et al. 1990; Clapperton et al. 1994). This outstandingly quick succession between competing *Vespula* species has been arguably explained by superior foraging capabilities of *V. vulgaris* (Harris et al. 1994).



Fig. 1.7 *Vespula* wasp forager taking flesh from a stranded anchovy (*Engraulis* sp.), found still alive while patrolling the sea water edge (Cannitello, Southern Italy, July 2013).

1.5 Information transfer and coordination among wasp foragers

The sharing of information among individuals can be considered one of the driving forces that led to the evolution of animal societies (Wagner and Danchin 2003). The ecological success of insect societies has been commonly linked to their ability to work with an apparent unity of purpose (Wilson 1971, Johnson 2010). One of the most studied group tasks in social insects is recruitment for food retrieval, after an individual discovers a food source that is much larger than what can be handled alone (Jaffe et al. 2012). In the context of foraging, “recruitment” (*sensu stricto*) is defined as a specific communication, mediated by signals (stimuli shaped by natural selection to convey expressly the information, in this case beneficial both to the sender and the receiver (Lloyd 1983; Nieh 2009)), that serves to bring nestmates to a food source (Wilson 1971).

Social wasps are the only group of eusocial insects in which there has been no previous demonstration of nest-based foraging recruitment (Raveret-Richter 2000; Jeanne and Taylor 2009; Nieh 2009). Naumann’s (1970) anecdotal report of a “departure dance” in *Protopolybia pumila* is unique. Intranidal (within-nest) or at nest communication of food location is undocumented (Raveret-Richter 2000; Jeanne and Taylor 2009; Nieh 2009). The giant hornet *Vespa mandarinia* is the only wasp known to utilize field-based signals for foraging purposes (Jeanne and Taylor 2009). The possibility of foraging communication was investigated in the wasps *Agelaia* and *Polybia*, which are known to use pheromones for swarming, but no evidence of foraging signalling was found (Hrncir et al. 2007; Schueller et al. 2010; Taylor et al. 2011; Schueller and Jeanne 2012). Hence, the traditional view is that foraging in social wasps is an individual activity.

Without signals and communication, food information transfer can take place via cues, both in the field and at the nest (Nieh 2009). Several studies have found forms of coordination in social wasp foraging behaviour (Taylor 2012). For example, local enhancement occurs when an animal’s attention is directed to a particular location or object by the action or presence of conspecifics (Raveret-Richter 2000), and several studies have observed this phenomenon in social wasps, including vespulids. Evidence for foraging activation (an increase in the probability of an individual leaving the nest as a result of information received from successful foragers) was found in the social swarm-founding wasp, *Polybia occidentalis* (Hrncir et al. 2007). In other social wasp species (*V. germanica*, *Protopolybia pumila*, and *P. occidentalis*), an increase in departure rates of foragers was observed to be triggered by the simple insertion of a sucrose solution inside the nest activation, demonstrating that food-related cues are sufficient to elicit some form of group response (Overmyer and Jeanne 1998; Jandt and Jeanne 2005; Schueller et al. 2010; Taylor et al. 2010). In social wasps, hence, there is no evidence of nest-based foraging communication, and information appears to circulate via cues (passive sources of information, considered evolutionarily basal (Nieh 2009; Johnson 2010)).

1.6 Thesis aims

There is a clear gap in our understanding of fundamental features of insect societies, such as the division of labour among workers and their specialization. Moreover, our knowledge of social wasps is limited, when compared to other social insects such as bees and ants. Among social wasps, the relatively few, closely-related yellowjacket species are notable for their emerging differences in social and foraging biology. My work represents a contribution to our understanding on the complexity and variability within insect societies. I have used the common wasp *Vespula vulgaris* (L.) as a study model, and I have investigated to what degree wasp workers from the same colony can vary in fundamental behavioural traits, such as activity and aggressiveness. I have focused my attention on individual wasps of known age and size, studying their behaviour over their entire lives, investigating polyethism and elitism within these wasp societies. I have also explored a debated topic in this group of social insect, the coordination of wasp workers in foraging activities.

In Chapter 2, I investigate the possibility of recruitment in the common wasp, testing the hypothesis that wasps share food-related information inside the nest and show foraging activation. Is the scent associated with a food resource brought into the nest by successful foragers learned and used by nestmates in their foraging choice in the field? Is there any evidence of information transfer concerning the location of food? Can a *V. vulgaris* colony modulate its foraging effort on the basis of intra-nest social information flow?

Chapter 3 focuses on the intra-colonial variability in foraging activity, task performance, and survival among *V. vulgaris* workers. Individuals of known age and size were studied throughout their entire life to answer the following questions: to what degree do wasp nestmates differ in their foraging activity? Do specialized and elite wasp workers exist? How can individual foraging patterns

be related to age and size? What is the relationship between foraging activity, individual size and survival?

In Chapter 4, I test the hypothesis that specialist foragers are more efficient than generalists within the same colony. Are specialist wasps performing more trips? Are their trips accomplished in relatively shorter time? The possibility that specialists and generalists show differential life expectancy is also tested.

Chapter 5 is concerned with quantifying intra-colonial variation in aggressiveness, measuring the sting extension response (SER) of wasps responding to a mild electric shock and using the SER as a proxy for individual aggressiveness. Is the individual SER plastic, changing throughout wasps' life? Is the SER linked to wasps' age or size? Are individuals defending the nest showing higher SER scores? Is the SER proportional to the intensity of the negative stimulus?

Finally, in Chapter 6, I summarize and synthesize the main findings of my thesis, outline the constraints encountered and discuss opportunities for further research.

Chapter 2

Nest-Based Information Transfer and Foraging Activation in the Common Wasp (*Vespula vulgaris*)

2.1 Abstract

The apparent absence of intra-nest signals and communication about food resources (recruitment) among social wasps does not rule out the possibility of information transfer and coordinated foraging among nestmates. In the present study, we tested the hypothesis that the common wasp (*Vespula vulgaris*) shows nest-based information transfer and foraging activation: an increase in the probability of an individual leaving the nest as a result of information about resources received from successful foragers. We controlled for the possibility of local enhancement, chemical trails at the food source and climatic variation. We found evidence that food choice and discovery of resources in the field by naïve foragers was assisted by information previously or simultaneously provided by experienced nestmates. This information was related to chemical cues associated with the food and possibly to its location. Our observations suggest piloting between common wasp foragers. At the trained nest, there was a change in foraging effort at the colony level when known resources were available. Reactivated, experienced foragers were the main group responsible for the increase in foraging traffic rate observed at the colony level. To our knowledge, this is the first study clearly demonstrating nest-based information transfer about food resources in *V. vulgaris* and one of the few providing evidence of foraging activation in social wasps. Our data are consistent with the possibility of recruitment in this group of social insects.

Keywords Recruitment, Social information, Foraging activation, Associative learning, Foraging traffic rate, Pilot flights

2.2 Introduction

Information can be acquired by individuals directly (personal information), or indirectly, by gathering it from other individuals (social information) (Dall et al. 2005). Useful information can be available to individuals in the form

of cues or signals (Wilson 1971). Cues are passive sources of information, aspects of the physical or social environment that may convey information incidentally, and are considered evolutionarily basal (Nieh 2009; Johnson 2010). Signals are stimuli shaped by natural selection to convey expressly the information, which can be actively targeted to their recipients (Nieh 2009, Johnson 2010). Stimuli can be transmitted through different channels such as visual, chemical, acoustic or thermal (Nieh 2009; Jaffe et al. 2012).

The transfer of information through signals is “communication” (Wilson 1971). In social insects, “recruitment” is defined as a specific type of communication that brings nestmates to a location where work is required (Wilson 1971). In the context of foraging, this communication serves to bring nestmates from the nest to a food source (Wilson 1971). Recruitment can be costly (Dechaume-Moncharmont et al. 2005), situation-dependent (Jeanne et al. 1995), and its intensity and efficiency can vary dramatically among species (Aguilar et al. 2005; Jarau et al. 2000). In ants and termites, recruitment is well documented, and is frequently mediated by pheromone trails (Jaffe et al. 2012). Inside their nests, bumble bees and stingless bees use a variety of foraging signals via distinct information channels (Biesmeijer and Slaa 2004; Dornhaus and Chittka 1999, 2001, 2004; Nieh 2004). Some stingless bees show evidence of “piloting” (analogous to the ants’ “tandem running”), in which one individual leads one or more nestmates to a resource (Aguilar et al. 2005, Nieh 2009). Honey bees are well known for their dance communication, codifying resource profitability and location relative to the hive (von Frisch 1967).

Despite some reports (Naumann 1970; Taylor 2012), there is no clear evidence that social wasps (Hymenoptera: Vespidae) use signals to recruit nestmates to food sources (R. L. Jeanne & Taylor, 2009; James C. Nieh, 2009; Raveret Richter, 2000). The giant hornet, *Vespa mandarinia*, is the only wasp known to utilize a field-based recruitment signal. In this species, individual scouts spotting beehives scent-mark them by means of a pheromone. Scouts trigger a group attack, coordinated with a band of nestmates (Ono et al. 1995).

Thus, in social wasps, active information transfer seems to be the exception, where individual exploitation of environmental cues have been suggested to be the rule (Raveret-Richter 2000).

Social wasps are typically generalist and opportunistic foragers that use a variety of mechanisms to locate and choose the resources needed (Raveret-Richter 2000; D’Adamo and Lozada 2009; Lozada and D’Adamo 2011). Individual wasps are influenced by past foraging experience and have the ability to learn landmarks through orientation flights and to promptly associate colours and odours with food rewards (Raveret-Richter 2000). Foragers integrate old and new memories (D’Adamo and Lozada 2009) and are capable of generalizing visual stimuli (Lozada and D’Adamo 2011). In general, foraging wasps utilize a combination of cues to obtain the greatest amount of information (Jeanne and Taylor 2009). Landing responses are elicited mainly by odour cues on both protein and carbohydrate resources (Moreyra et al. 2006). Using their learning abilities, social wasp foragers return to foraging sites where they have been successful and may feed repeatedly on the same kind of resource, thus acting as facultative specialists (Raveret-Richter 2000).

Interesting insights can come from the comparison between social and solitary wasps, showing facultative specialization on a finer scale (e.g. prey genus within the constrained order or family of prey) (Gonzaga and Araújo 2007, Santoro et al. 2011). Individual specialization in solitary wasps is driven by many factors, including predator/prey size relationships (Polidori et al. 2010), prey mobility (Polidori et al. 2013), abundance (Santoro et al. 2011) and nest-nest distance (Polidori et al. 2012). The ultimate availability of the resources and the inter-individual information flow, together with the underlying learning processes involved, can be key for explaining individual foraging patterns both in a solitary and a social context.

Although the traditional view is that foraging in social wasps is an individual activity (Raveret-Richter 2000), several studies have highlighted that cue-mediated forms of information transfer and co-ordination appear to occur amongst

foragers (e.g. Overmyer and Jeanne 1998; Schueller et al. 2010; Taylor 2012). In the German wasp, *Vespula germanica*, and in *Polybia occidentalis*, scent extracts diluted in sucrose presented in training feeders, or directly inserted in the nest, were associated with food by naïve wasps and used as cues to focus attention on resources outside the nest (Overmyer and Jeanne 1998; Jandt and Jeanne 2005; Taylor et al. 2010; Schueller et al. 2010; Taylor et al. 2011; Taylor et al. 2012a; Taylor et al. 2012b). This food-related transfer of information may take place in the field (e.g. local enhancement) and at the nest (e.g. foraging activation) (Nieh 2009).

Local enhancement occurs when an animal's attention is directed to a particular location or object by the action or presence of conspecifics (Raveret-Richter 2000). This phenomenon is well known in social wasps: foragers frequently show a non-random, aggregated distribution both on carbohydrate and protein resources (R. L. Jeanne & Taylor, 2009). Individuals of species such as *V. vulgaris* and *V. germanica* clearly show a tendency to aggregate (Raveret-Richter and Tisch 1999). This phenomenon is cue-based, not requiring any active signal (Parrish and Fowler 1983), and is context dependent (Raveret-Richter 2000, Wilson-Rankin 2014). Indeed, wasps of the genus *Vespula* are not known to scent mark food sources (Jandt et al. 2005, Taylor et al. 2011). Yet, chemical trails (e.g. cuticular hydrocarbons footprints) might still play a role in the context of wasp foraging (Raveret-Richter 2000, Jeanne and Taylor 2009), as they do in individual intra-nest orientation (Steinmetz and Schmolz 2003, Steinmetz et al., 2002), and the search for nest-sites of swarm-founding species (Naumann 1975; Taylor et al. 2011).

Foraging activation consists of an increase in the probability of an individual leaving the nest as a result of information received (at the nest) from successful foragers (Nieh 2009). Accumulation of *Vespula pensylvanica* foragers at baits in the field was greater when repeated visitation by nestmates was allowed (Wilson-Rankin 2014). Hrncir et al. (2007) demonstrated that *P. occidentalis* foragers only arrived at feeders after nestmates were trained to those feeders. An

increase in forager departure rates can be artificially triggered in *P. occidentalis* and *V. germanica* colonies by the simple insertion of a sucrose solution inside their nests (Taylor et al. 2012a, b), as is also known for honey bees and bumble bees (Dornhaus and Chittka 2001). Cues alone are hence sufficient to modulate social wasp colonies' foraging activity.

The common wasp *Vespula vulgaris*, native to Eurasia, has become a notorious pest in countries such as Argentina and New Zealand, attaining high densities and causing major ecological impacts in the invaded range (Lester et al. 2014). In the New Zealand beech forests (*Fuscospora* and *Lophozonia* spp.), the common wasp has spectacularly displaced the German wasp within a few years of invasion (Harris et al. 1994). To our knowledge, no experiment has yet demonstrated nest-based foraging information sharing in *V. vulgaris*. Moreover, no study to date has investigated if social information flow coming from wasps freely foraging in the field can trigger variation in foraging effort measurable at the colony level. Both issues are worth investigating, considering the plasticity of foraging ecology and interspecific variation known within the genus *Vespula* (e.g. Parrish et al. 1983; Raveret-Richter and Tisch 1999; D'Adamo et al. 2001; Kim et al. 2007; Grangier and Lester 2011). We have hence designed an experiment to study the possibility of intra-nest information sharing and foraging activation in the common wasp, aiming to answer the following questions:

I) Does *V. vulgaris* show nest-based social information sharing? Controlling for local enhancement and chemical cues potentially left at the food source, is the scent associated with a food resource simultaneously or previously brought into the nest by successful foragers learned and used by nestmates in their foraging choice in the field? Is there any evidence of information transfer concerning the location of food?

II) Can a *V. vulgaris* colony as a whole change its foraging effort on the basis of intra-nest social information flow? Is the renewed availability of a

specific food source related to a change in the foraging activity of a colony containing individuals familiar with that food source? What is the role of individual experience in the eventual increase in the foraging traffic rate at the nest?

2.3 Materials and methods

2.3.1 Wasp colonies and study site

Two underground colonies of *V. vulgaris* were excavated from Arthur's Pass, New Zealand (42°59'20.63"S, 171°44'48.79"E), on 10/02/2012. The nests were placed in wooden nest boxes (60×38×35 cm inside dimensions) with a metal grid 2×2 cm wide suspended inside at mid-height. The nest boxes were placed in the grounds of a Plant & Food Research Ltd. laboratory in Lincoln. The two nests were placed 240 m apart from each other. Most of the site was covered in mown grass, with several tree patches dominated by *Quercus* spp. Nest box entrances were then opened and wasps were allowed to forage outside of the nest boxes via a clear plastic tube (20 cm length, 2 cm inside diameter). The boxes had a glass wall covered with a wooden sliding door that allowed us to monitor the status of the nests, which grew in size during the season. Colonies were given three weeks to recover and acclimate before our experiments began. Nest 1 was used as the experimental colony, in which part of the workforce ("trained foragers") was familiar to a particular feeding location ("training site") and to scented sucrose, and information about food source (food scent and location) could be potentially shared among nestmates. Nest 2 was used as the control colony.

2.3.2 Individual foraging choice (in the field)

Training protocol

One group of wasps from Nest 1 was trained to visit one feeding station ("training station") in a specific location ("training site"). On 1st of March 2012, training was commenced near Nest 1 by allowing wasps to feed from a piece of

tissue paper soaked with a 30% sucrose solution kept directly in front of the nest box entrance. The tissue with the feeding individuals on it was then transported manually to the training feeding station (Fig. 2.1), initially placed 1 m from the nest. The procedure was repeated several times to encourage the wasps to become familiar with the artificial feeder. The training station was then moved in 5 m steps away from the nest at intervals of approximately 15 min, until the desired final location, the “training site” (Fig. 2.1), 60 m north-east of the nest. At this point, the sucrose solution was scented with 3 ml/l vanilla extract (Hansells Natural Essence, Hansells Food Group, Auckland) (experimental series I).

To ensure that all the individuals directly experiencing the training site and scent were recognizable, all the wasps visiting the feeder were marked with water-proof, non-toxic FasTM orange paint (Fine Art Supplies Ltd, Auckland) applied with a brush on their thorax and abdomen while they were feeding (referred to as “experienced foragers”). As noted previously (e.g. Wilson-Rankin 2014), marking in situ (without constriction or anaesthesia) does not appear to disturb foragers. Exposure of the scented food and concomitant marking was done for the next four hours and during 2 and 4 March (an additional six hours). The station was removed and re-presented in the same location each time. From the 12 March, the training station was re-presented providing a sucrose solution scented with 3 ml/l peppermint extract (Hansells Natural Essence) (experimental series II). Experienced foragers landing on the station were re-marked with FasTM green paint. The odour switch was made to control for any innate odour preferences potentially biasing choices made at the choice station (see below).

2. Information Transfer and Foraging Activation

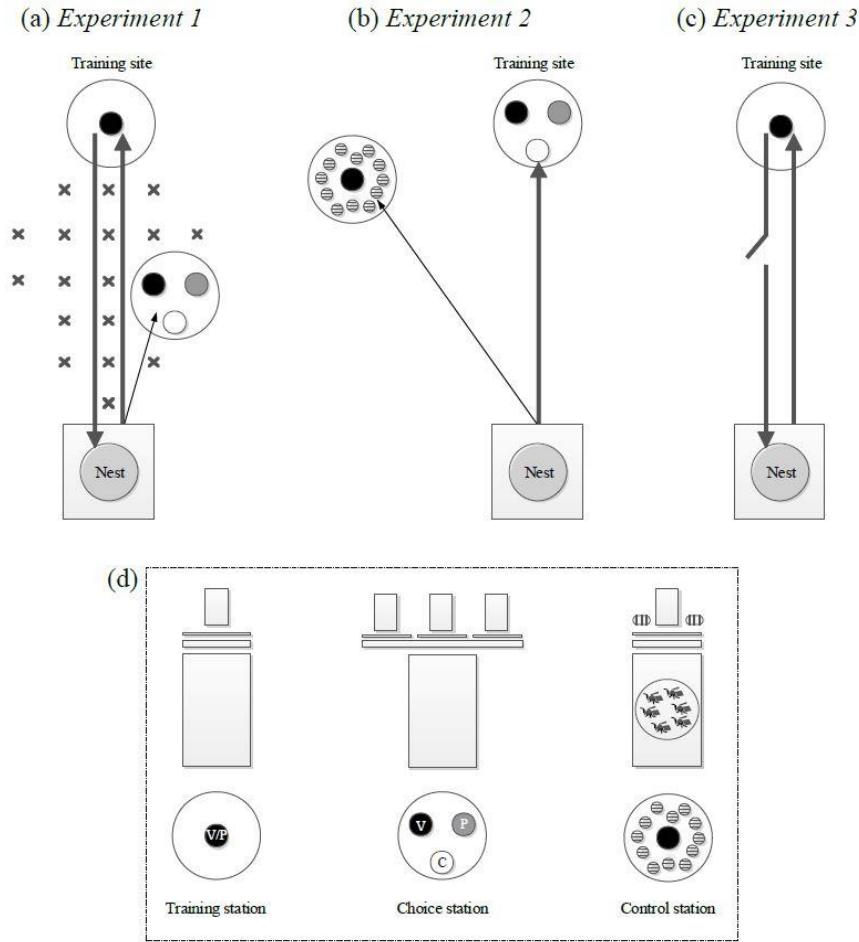


Fig. 2.1 Plan view of the experimental set up for (a) experiment 1, (b) experiment 2, (c) experiment 3 and (d) detail of the feeding stations (above, side view; below, plan view). (a) Experiment 1 – Foragers visiting the training site were free to go back and forth from the nest (no capture at the training site: social information allowed – see arrows). (b) Experiment 2 – Foragers visiting the training site were unable to go back and forth from the nest (capture at the training site: social information not allowed – see arrows). (c) Experiment 3 – Foragers visiting the training site were alternatively captured or not. At the beginning of each trial, capture for the whole duration of the trial was decided flipping a coin (alternate social information– see arrows). (d) The training station had one feeder consisting of a glass jar (18 cm high, 10 cm diameter) containing a 30% sucrose solution scented with vanilla (V, series I) or peppermint (P, series II) placed inverted over a white plastic dish (26 cm diameter) containing four layers of folded tissue paper. The feeder was placed on the top of a white PVC cylinder (40 cm high, 30 cm diameter). The choice station had a rotatable array with three feeders separated by 10 cm from each other. One feeder was scented with vanilla (V), another with peppermint (P) and a third one had no scent (C). Sugar and scent extract concentration were the same as the training station. The position of the choice station was variable: it was placed in the area between Nest 1 and the training station (locations marked by “x”, experiment 1) or instead of the training station at the training site (experiment 2). The control station was built as the training one, but the feeder was characterized by the presence of 60 painted pins (effective wasp dummies – Parrish and Fowler 1983) on the plastic dish. The cylinder sustaining the feeder had a transparent band and four holes (8 cm diameter), each one covered by a thin metal mesh. Inside the cylinder, a visible and variable number of wasps (15 to 37) kept in individual transparent cages were feeding on pads imbibed with sucrose solution.

Experimental protocol

Experiment 1 – Newcomers’ arrival and choice with simultaneous intra-nest social information

In this experiment, the training and the choice station were presented simultaneously (Fig. 2.1a), with two observers sitting 1 m from each station. The training station was presented at the training site (see above). The position of the choice station, always closer to Nest 1 than the training one, was changed every hour and was determined randomly from among 19 intersections of a 10×10 m imaginary grid (Fig. 2.1a, see “×”).

At the training station (Fig. 2.1d), wasps were not captured and were free to fly back and forth between the training site and the nest (“social information allowed”). All unmarked, naïve individuals landing on the training station (referred to as “newcomers”) were marked with Fas water-based paint on thorax and abdomen while feeding (orange in series I; green in series II) and the number of newcomers was recorded at 10 min intervals.

The choice station (Fig. 2.1d) was designed to test whether the choice of the visiting wasps was influenced by the scent associated with resources brought home by nestmates and hence represented a test for nest-based information transfer. On this station, the exact arrival time and the choice of each wasp (first feeder on which each individual landed and fed) was recorded. To avoid experimental artefacts due to a side preference of the individuals (Scheiner et al. 2013) or wind direction (Overmyer and Jeanne 1998), the feeders’ position relative to the choice station was changed randomly by rotating the station’s array every 10 min. Both experienced foragers and newcomers arriving at the choice station were captured with 45 ml specimen jars, so that individual choice was not driven by visual cues (the use of chemical footprints was ruled out by Control experiment 1, see below). Experienced foragers (whose choice was not included in the analysis) were released at the training location at the end of each trial. Newcomers were later anesthetized with carbon dioxide, individually marked and, once they recovered, released in front of the Nest 1 entrance. Marking was achieved

by means of a colour and position code of paint spots applied with water-based, soft felt-tip pens (Unipaint marker; Mitsubishi Pencil Co., Tokyo, Japan) on the thorax and one white Fas paint spot on the abdomen. If the newcomers were seen entering the nest box immediately or during the following days, they were considered part of the trained colony and included in the analysis on the feeders' choice (see below). Experimental trials ($n=11$) lasted between 60 and 200 minutes, encompassing a total of 31 h.

Experiment 2 – Newcomers' arrival and choice with past intra-nest social information

In this experiment, the choice and the control station were presented simultaneously, at the same distance from Nest 1 (Fig. 2.1b). The choice station was placed at the training site, the control station 60 m north of Nest 1 and 30 m west of the training station.

The control station (Fig. 2.1d) was designed to give wasps all the cues provided by a group of other foraging individuals to control for potential local enhancement biasing the number of newcomers arriving at the two stations (Fig. 2.1b). All the wasps visiting both stations were captured as soon as they landed on the feeders ("social information not allowed"). At the choice station, the choice of each individual was recorded. Experienced foragers were captured and released at the end of the trial. Newcomers were captured, individually marked and released after the trial, following the same protocol described in experiment 1 for the wasps arriving at the choice station. The choice station's array (Fig. 2.1d) was rotated every 10 minutes. Experimental trials ($n=3$) lasted between 120 and 240 minutes, encompassing a total of 10 hours.

All the experiment 1 and 2 trials were run between 14.00 and 1.00 h. Data in series I (training scent = vanilla) were gathered between 5 and 12 March (12h), while data in series II (training scent = peppermint) were gathered between 12 and 26 March (29h).

Experiment 3 – Newcomers’ arrival with alternate social information

In this experiment, we tested whether more naïve wasps arrive to a feeder when simultaneous social information is available at the nest. The training station was placed at the training site and incoming wasps were alternatively captured or not, following a random sequence in the trials (Fig. 2.1c). Counts and marking of newcomers were performed following the same protocol described in experiment 1 for the wasps arriving at the training station. Data were collected between 26 March and 12 April. Experimental trials ($n=11$) lasted between 60 and 180 minutes, encompassing a total of 28 hours (capture, 17h; no-capture, 11h).

Control experiment 1 - Innate odour preferences, eventual food-site marking substances

To control for innate odour preferences, the training and control scent utilized during series I was swapped during series II. The plastic dishes and the tissue paper of the feeding stations were changed daily. To control for eventual food-site marking substances, the plastic dish and tissue paper were changed between trials and every day. Moreover, one feeder with non-scented sucrose solution was kept in front of Nest 2. The feeder was visited by foragers from the untrained colony (Nest 2) and later removed and replaced for four 30 min intervals with the experimental feeder soon after experiment 2 trials (and other wasps’ visits). All the wasps landing on the feeders were captured and their choice recorded. Individuals were kept in small cages and used in the control station without being released, to ensure that only individuals naïve to the scents were counted during each trial.

Control experiment 2 - Detection of the feeder by individual search

Before the beginning of the training phase, we placed the training station (unscented) in its final location, for four consecutive days. During each day (7 am – 9 pm) we observed hourly the feeding station to see if wasps were foraging on it. No wasps were observed on the feeding station over these four days.

2.3.3 Colony foraging effort (at the nest)

To measure colony foraging effort and assess colony size during the experiment, we measured the foraging traffic rate (FTR) of the nests. FTR, here defined as the total number of wasps entering and exiting the nest in a 10 minute interval, was recorded daily by means of direct observations and video analysis. During the previously described experiments, two cameras were set on the top of Nest 1 and Nest 2 tube entrances. Video recordings also allowed determining the provenience of the wasps individually marked at the feeding stations. For eight days, simultaneously to experiment 1 trials, Nest 1 and Nest 2 FTR was video-recorded. On each day, the cameras filmed the nest entrances for the first ten-minute interval of each hour starting when the feeding stations were first placed in the field (Interval I) and then during the trials, after one (Interval II) and two hours (Interval III), respectively. The estimated number of workers in the two colonies was obtained adapting Malham's et al. (1990) predictive equation based on foraging traffic rate ($\text{number of workers} = 3.2243 \times \text{FTR}$). For each colony, an average FTR value was obtained from observations over the entire duration of the experiments.

2.3.4 Analysis

Fisher's exact test was used to analyse the wasps' choice on the choice station i.e. the differences in frequency between the numbers of newcomers from Nest 1 landing on the training scent vs the control scent dish and the control scent versus the unscented dish (experiments 1 and 2). We further tested whether individuals from Nest 2 (where no foragers were trained with scented sucrose) choose the feeders in equal proportions, as expected for control trials.

To test for significant variations in the FTR of Nest 1 and Nest 2 during experiment 1, linear mixed effects models were used. We tested the effect of the time interval on the number of wasps (all wasps from Nest 1 and 2, experienced and naïve individuals from Nest 1), with date as a random effect and climatic

parameters (temperature, wind speed and direction) as covariates. Linear mixed effects models were also used to test for differences in the number of newcomers arriving at the training site vs other sites during experiment 1 and experiment 2 and newcomers arriving at the training site during experiment 3 trials. We tested the effect of site and trial on the number of newcomers arriving at the stations in one hour periods, with the date as a random effect and climatic parameters (temperature, wind speed and direction) as covariates.

Ten minute-interval climate data were obtained from the NIWA/Plant & Food Research meteorological station (id: 17603), 1km north from the experimental area (data accessed from the National Climate Database, <http://cliflo.niwa.co.nz/>). A summary of the climatic parameters measured during the trials and considered in the analysis is available in the supplementary material (Table 2.S1).

Data analysis was performed using R 3.0.2 (R Development Core Team, 2013).

2.4 Results

During the experimental trials, Nest 1 had an estimated average number of 1636 (± 357) workers, Nest 2 1383 (± 323) workers. The unscented training station, placed at the training site for 48 h before the training phase, was not discovered by any wasp. At the end of the first training phase, 180 individuals (experienced foragers) were marked at the training site. No marked individuals were seen entering Nest 2 during the experiment. During the experimental trials, a maximum of 95 individuals were simultaneously present on the training station. At the choice station, during experiments 1 and 2, 81 newcomers from Nest 1 arrived (see below). We could not ascertain the provenience of eight individuals, which were not included in the analysis. No marked wasps were seen departing or arriving from Nest 2.

2.4.1 Individual foraging choice (in the field)

Newcomers' choice with intra-nest social information

Our first hypothesis was that naïve foragers from Nest 1 would show a preference for the feeder scented the same as the most recent resource brought into the nest by experienced foragers (i.e. the training scent) and that more newcomers would arrive when experienced nestmates were not captured at the training site. We expected no preference from naïve foragers of Nest 2. For Nest 1, there were highly significant differences ($P < 0.001$) in the numbers of wasps choosing the three feeders on the choice station, with naïve foragers preferentially choosing the training scent simultaneously (experiment 1) or more recently presented (experiment 2) at the training site, regardless of whether the training scent was vanilla and the control scent peppermint (as in series I) or vice versa (series II) ($\chi^2_2 = 0.18$, $p = 0.672$). No difference was found among individuals from Nest 2 ($P = 0.339$, Table 2.1). There was no difference between the number of wasps at feeders with control scent versus no added scent ($P \geq 0.335$, Table 2.1).

Table 2.1 Feeding choice of naïve *Vespula vulgaris* foragers (newcomers) from the trained NEST 1 (experiment 1, 2) and untrained NEST 2 (Control) at the choice station. In series I, the training scent was vanilla, the control scent peppermint. In series II, the training scent was peppermint, the control scent vanilla. Odds-ratios and P-values are the results of a Fisher's exact test on the relevant 2×2 contingency table.

NEST 1	Experiment	Series	Number of naïve foragers choosing:			training vs control scent		control vs no scent	
			training scent	control scent	no scent	odds-ratio	P (df=1)	odds-ratio	P (df=1)
	1	I	30	5	2	10.339	<0.001	3.455	0.335
		II	11	6	4				
		total	41	11	6				
	2	I	3	0	1	Inf	<0.001		
		II	19	0	0				
		total	22	0	1				
NEST 2	Control	I	23	19	22	1.490	0.339	1.012	1.000
		II	42	34	29				
		total	65	53	58				

Newcomers' arrivals at the feeding stations

During experiment 1, a total of 263 naïve individuals arrived at the training station. At the choice station, 58 newcomers arrived from Nest 1 (Table 2.1), five were of unknown provenience. During experiment 2, a total of 23 newcomers arrived at the choice station (Table 2.1), three of which were of unknown provenience. No newcomer arrived at the control station. More naïve wasps arrived at the training site compared to other sites (Fig. 2.2), both during experiment 1 ($t=5.710$, $df=47$, $p<0.0001$) and experiment 2 ($t=4.532$, $df=9$, $p<0.01$). None of the climatic parameters that we measured significantly influenced the number of unmarked wasps arriving at the stations during these experiments. During experiment 3, a total of 94 naïve individuals arrived at the training site. Most of the naïve wasps arrived to the feeding station when experienced foragers were not captured ($t=8.230$, $df=13$, $p<0.0001$, Fig. 2.3), and more arrived with higher wind speed ($t=3.800$, $df=13$, $p<0.01$).

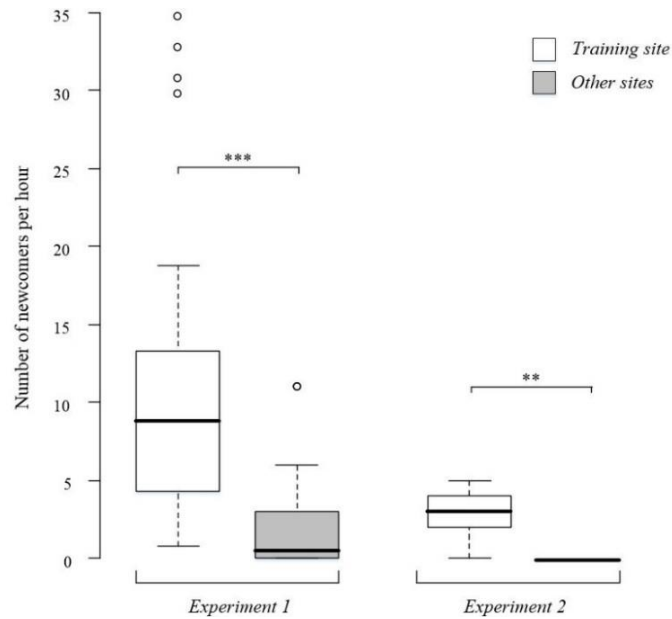


Fig. 2.2 Number of newcomers arriving at the feeding stations during experiments 1 (31h) and 2 (10h). Boxes represent the lower and upper quartile, the bold line is the median and whiskers represent extreme values, with the circles identifying outliers. We tested the difference in numbers of newcomers arriving at the training site vs other sites in experiment 1 and 2. *** $p<0.0001$, ** $p<0.01$.

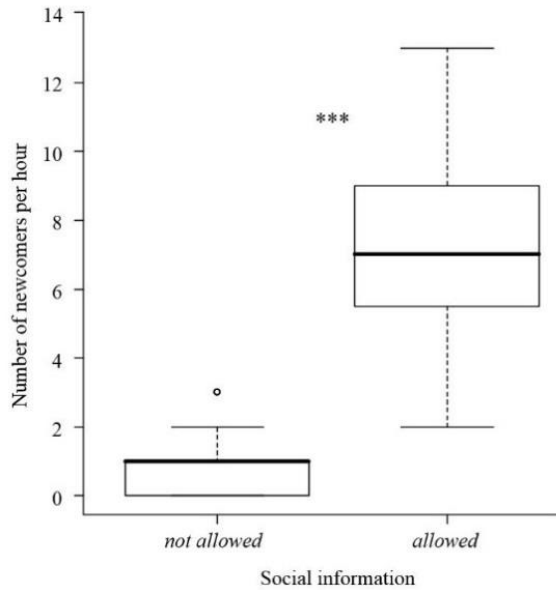


Fig. 2.3 Number of newcomers arriving at the training site during experiment 3 (28h). Boxes represent the lower and upper quartile, the bold line is the median and whiskers represent extreme values, with the circles identifying outliers. We tested the difference in numbers of newcomers arriving at the feeding station between trials in which social information was not allowed (experienced foragers were captured) vs allowed (experienced foragers were not captured). *** $p < 0.0001$.

2.4.2 Colony foraging effort (at the nest)

Our second hypothesis was that only Nest 1, with trained, experienced foragers, would show an increase in the foraging traffic rate when the feeding stations were placed in the field.

During experiment 1 trials, FTR of all individuals varied significantly in Nest 1, but not in Nest 2 (Fig. 2.4). Comparing all the individuals from Nest 1, there was an increase in traffic one hour after the positioning of the stations in the field (time interval II vs I), with FTR returning to the initial intensity after two hours (time interval III vs I) (Fig. 2.4). Among the climatic parameters, wind speed influenced overall FTR of the colony ($t=2.825$, $df=11$, $p=0.017$). Considering naïve individuals from Nest 1, there was a marginal increase in FTR measured in interval II and a significant decrease in interval III (Fig. 2.4). The linear mixed effects model highlighted a negative effect of wind speed on the traffic rate of these individuals ($t=3.167$, $df=11$, $p=0.009$). Considering experienced individuals from Nest 1, FTR values were higher both during interval II and III than during interval I (Fig. 2.4). Details of the analysis are available in the supplementary material (Table 2.S2).

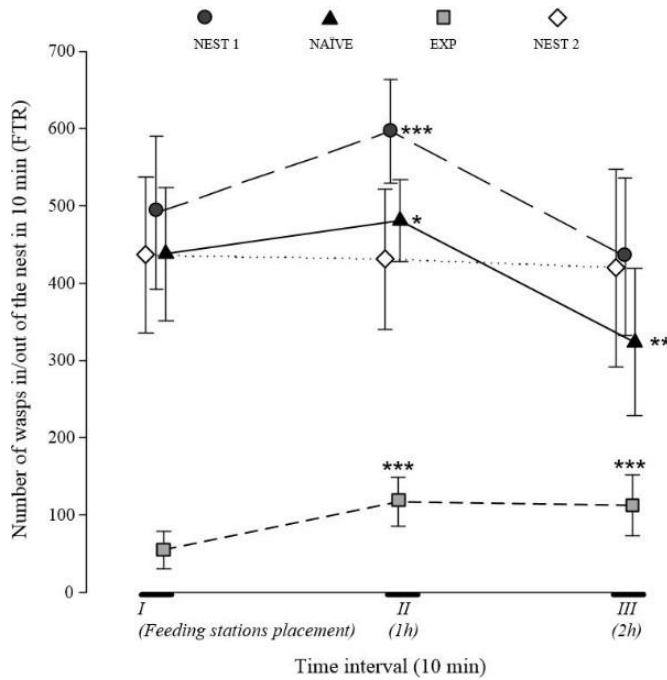


Fig. 2.4 Foraging traffic rate (FTR) measured over time during experiment 1 trials for NEST 1 (trained) and NEST 2 (untrained, control). The FTR of NAIVE and experienced (EXP) foragers from the trained colony are shown separately and summed together (NEST 1). FTR values are mean \pm SE. During time interval I, the foraging stations were placed in the field. P values refer to the difference in FTR at interval II and III compared to interval I. *** = $p < 0.001$, ** = $p = 0.002$, * = $p = 0.048$ (Please see supplementary material, Table S2.2 for further detail).

2.5 Discussion

Our study provides, for the first time, evidence of intra-nest social information sharing in the common wasp, *V. vulgaris*. Our two experiments (1, 2) for food choice in the field, similarly to Overmyer and Jeanne's (1998) study on *V. germanica*, eliminated the confounding effect of local enhancement, which potentially biased Maschwitz et al.'s food choice results for *V. vulgaris* and *V. germanica* (1974). The newcomers from the experimental colony were choosing resources matching those brought into the nest by successful foragers (the experienced individuals) (Table 2.1). Hence these individuals must have learned inside the nest to associate the scent added to the sucrose at the training station to the food shared by their nestmates and used this information, a chemical cue, to find food resources in the field. Our results on the food choice of naïve *V. vulgaris* foragers parallel similar results on *V. germanica* (Overmyer and Jeanne 1998). Trophallaxis represents one possible mechanism for achieving this transfer of information, as discussed for other wasp species such as *V. germanica* (Overmyer and Jeanne 1998, Jandt and Jeanne 2005, Taylor et al. 2012a, 2010, Taylor and Nordheim

2011) and *P. occidentalis* (Schueller et al. 2010). The role of trophallactic exchanges in foraging information transfer and associative learning was demonstrated in bees and ants (Farina et al. 2005, Farina 1996, Provecho and Josens 2009).

Interestingly, the sucrose with the training scent was preferentially chosen by naïve foragers from the experimental colony both when the social information flow was concurrent (experiment 1) or up to several days prior (experiment 2). This finding is consistent with the fact that scents of rewarding foods are stored in long term memory by foraging hymenopterans (Jandt and Jeanne 2005). Indeed, wasps are well known for their prompt associative learning (Raveret-Richter 2000). Remarkably, vespids foragers are able to integrate old and new experiences after one or very few learning episodes (Weiss et al. 2004; Lozada and D’Adamo 2011). Our set-up also provides evidence that additional odours, unless linked to previous experience, are meaningless to vespulids (Table 2.1), supporting Taylor’s et al. (2012a) findings on *V. germanica*.

During experiments 1 and 2 (Fig. 2.1a, b), significantly more unmarked individuals arrived at the feeding station placed at the training site compared to the other station simultaneously presented in the field (Fig. 2.2). This was the case even if the latter was closer to the training colony (experiment 1, Fig. 2.1a, Fig. 2.2). During experiment 3 (Fig. 2.1c), more naïve foragers arrived at the same site when experienced foragers were free to travel back and forth from the experimental colony and social information was allowed (Fig. 2.3), ruling out the possibility of site biases (Nieh 2004). These results differ from work with *V. germanica* (Overmyer and Jeanne 1998) but are similar to Wilson-Rankin’s (2014) findings on *V. pennsylvanica*. Most excitingly, our data are consistent with the hypothesis of a mechanism of food location information transfer in the common wasp.

A proportion of the experienced foragers (36%) and newcomers (17%) were observed arriving at the training site almost simultaneously (within three seconds) with another individual. When the choice station was located at the training site (experiment 2), the prompt trapping of the first individual arriving

($n=8$) appeared to impede the landing of the following one. These tandem arrivals, neither observed nor described in other wasps (e.g. Overmyer and Jeanne 1998; Raveret-Richter 2000; Wilson-Rankin 2014), suggest pilot flights, as for other flying hymenopterans (Nieh 2004), with newcomers possibly finding their way to the training site by following experienced nestmates. Most newcomers arrived at the training site when experienced foragers were not captured (experiments 1 and 3) and the appearance of tandem arrivals could result from the high number of departing and arriving foragers at the feeding station. In fact, piloting is difficult to prove, but has often been suggested in stingless bees, on the basis of the temporal synchrony in the arrival times of foragers and recruits (e.g. Aguilar et al. 2005, Kajobe and Echazarreta 2005, Nieh 2009). We propose it as a possible mechanism partially explaining the observed patterns.

From this study, we temper our conclusions and urge caution regarding food location communication behaviours in this wasp species, for three main reasons. Firstly, to avoid site biases, training to different sites would ideally be required to confirm our results (Nieh 2004). Secondly, when not captured, a number of experienced foragers (most of them arriving during the first hour) were present on the feeder and could themselves have provided an additional, predominant cue to naïve foragers. Even if the experiment 2 control station was designed to control as much as possible for this potential local enhancement bias on the newcomers' arrival, no formal test for local enhancement has been standardized yet (Schueller et al. 2010). Thirdly, newcomers arriving at the training site during experiment 1 and 3 were not individually marked and we do not know how many of them were from the experimental colony (Nieh 2004), although we suspect it to be the vast majority. The disentangling of any mechanism underlying the observed patterns, such as piloting, was beyond the scope of the present study.

We also demonstrated that there can be significant adjustments in the foraging effort of one wasp colony in response to social information about one food source (Fig. 2.4). Given the difficulties encountered in the training of large

numbers of vespid foragers, our data are limited to one experimental colony and one control colony. Nonetheless, the experimental, trained colony as a whole repeatedly and consistently peaked in its foraging effort after the feeding stations were placed in the field (Fig. 2.4, interval II), while the control colony did not. We therefore interpret our results as evidence for a conditional, plastic response at the colony level to the availability of resources in the field. The rapid increase in foraging traffic rate of the trained colony, corresponding to an increase in the food brought back to the nest, is similar to that recorded by Taylor et al. (2012a) when inserting food in *V. germanica* nests. To our knowledge, we have demonstrated for the first time that a measurable colony response can be triggered by the activity of free-flying, trained wasps, without the insertion of the resource inside the nest.

Interesting dynamics emerged when considering naïve and experienced individuals' foraging effort separately (Fig. 2.4). When the feeding stations were present, a marginal and provisional increase in the foraging traffic of naïve individuals was recorded. The increase in traffic rate at the colony level was primarily due to an increased and stable response of reactivated, experienced individuals. In honey bees, when a forager flies back to the hive after a successful foraging trip, it transfers information about nectar odour and taste of the visited flowers without dancing, via simple trophallaxis (von Frisch 1967). In both honey bees' and stingless bees' colonies, successful foragers can stimulate a greater number of hive-mates to forage by sharing nectar with higher frequency (Farina 1996; Sánchez et al. 2004). If experienced bees resume their visits to known, previously exhausted nectar sources, a “foraging reactivation” takes place (Gil and Farina 2002; Sánchez et al. 2004). The experienced individuals can navigate back to the previously rewarding foraging sites through a “memory recall” mechanism (Reinhard et al. 2004). Similarly, successful bumble bee foragers bring home the odour of newly discovered food sources and actively alert nestmates, increasing colony foraging activity and conditioning resource choices of nestmates (Dornhaus and Chittka 1999, 2001, 2004). While experienced wasps maintained high

foraging activity, naïve foragers did not (Fig. 2.4, interval III). Indeed, these last individuals significantly reduced their activity. This result could be a homeostatic dynamic, resulting from task reorganization at the colony level. The strong resource influx due to the newly successful activity of experienced individuals would meet colony requirements and, with a necessary temporal delay, less successful naïve foragers may switch to other tasks (Johnson 2010).

Wind strength had an effect both on the trained nest foraging activity and on the number of naïve foragers arriving at the training site during experiment 3. The role of this environmental factor on the foraging activity of wasps and other flying hymenopterans was highlighted in the past (e.g. Blackith 1958, Comba 1999). Wind can indeed affect thermoregulation and flight costs, potentially impacting foraging ability and flight directionality (Comba 1999).

Our results, demonstrating learned associative preferences in food choice by “naïve” foragers, prove the existence of nest-based information transfer in *V. vulgaris*. The increase in foraging activity of the trained colony, in the presence of the feeding stations, provides evidence of foraging activation in the common wasp. These phenomena are likely cue-mediated, but active signals on different sensory channels are possible. Our observations on the arrival of newcomers at the feeding stations are indeed consistent with the possibility of communication and location-specific recruitment to food resources in social wasps, and suggest piloting as a possible foraging mechanism in the common wasp. Our findings, together with other studies on *Vespula* (e.g. Parrish & Fowler 1983, Harris 1994, Kim et al. 2007, Wilson-Rankin 2014), show interesting inter-specific differences within the genus, making generalizations difficult. We hence encourage comparative studies within the genus *Vespula* and further experiments to investigate the possibility of recruitment in social wasps.

2.6 Supplementary material

Table 2.S1 Summary [mean \pm SD (min, max)] of the climatic parameters measured during the experiments and included in the linear mixed effects models analysis performed.

	Climatic parameters		
	Temperature ($^{\circ}$ C)	Wind direction (degT)	Wind speed (mps)
Individual foraging choice (Experiment 1, 2)	16.3 \pm 2.7 (11.0, 23.2)	89 \pm 60 (5, 359)	4.6 \pm 1.9 (1.0, 10.2)
Newcomers' arrival (Experiment 3)	16.4 \pm 2.8 (12.5, 22.9)	75 \pm 55 (25, 196)	4.4 \pm 1.8 (1.4, 7.7)
Colony foraging effort (Experiment 1 trials)	15.2 \pm 2.1 (11.6, 19.0)	100 \pm 65 (10, 179)	5.0 \pm 1.8 (1.5, 8.6)

Table 2.S2 Results of the linear mixed effects models used to test for the effect of the time interval (I, II or III) on the number of wasps [all wasps from the trained nest (NEST 1) and the control nest (NEST 2), experienced (EXP) and NAÏVE individuals from Nest 1], with date as a random effect and climatic parameters (temperature, wind speed and direction) as covariates (Fig. 2.4, Table 2.S1).

Group	I vs II		I vs III	
NEST1	***	t=4.960, df=11, p<0.001	ns	t=-1.921, df=11, p=0.081
NAÏVE	*	t=2.230, df=11, p=0.048	**	t=-4.025, df=11, p=0.002
EXP	***	t=12.215, df=11, p<0.001	***	t=7.360, df=11, p<0.001
NEST 2	ns	t=-0.343, df=11, p=0.738	ns	t=0.126, df=11, p=0.902

2. Information Transfer and Foraging Activation



Chapter 3

The Common Wasp Radio-Tagged: Individuality, Polyethism by Age and Size, and Survival of *Vespula vulgaris* Foragers

3.1 Abstract

Social insect workers show impressive behavioural variation, but inter-individual differences have been traditionally overlooked and lost in the description of the “average worker”. This study focuses on the lifetime foraging careers of wasp workers from three colonies of *Vespula vulgaris*. We measured to what extent and how individual workers contribute to the different colony economies throughout their lives, assessing the role of wasps’ age and size, and how size and the age at the beginning of foraging activity affect individual life expectancy. We used radio-frequency identification technology to record the foraging activity of *V. vulgaris* individuals, from their first day of adult life to their death. Returning foragers were also video-recorded when passing the nest entrance with their loads and longitudinal data on their foraging task allocation were obtained. We found a striking individuality in wasps’ foraging behaviour. Many wasps (19–69%) never got to forage, and there was a huge variation both in life-time level of activity (elitism) and task performance (specialization) among foragers. Individual age and size partially explained the changes and variation in foraging behaviour (polyethism). Wasp foragers appeared to become more successful with age, performing more trips and carrying heavier fluid loads. Fluid collection increased with age and, between solid loads, pulp loads prevailed earlier, flesh loads later in life. Compared to smaller nestmates, bigger adults were more likely to become foragers and tended to start their activity earlier. Bigger foragers returning to the nest were observed more often with flesh loads, smaller individuals with no load. Workers starting foraging earlier died earlier. The dramatic differences found among individuals could be the product of weak selective forces at the individual level, due to the predominant colony-level selection. Extreme phenotypes and increased variability in key behavioural traits such as activity and boldness might be favoured in highly social species.

Keywords Hymenoptera, division of labour, elitism, specialization

3.2 Introduction

Animal societies, widely represented by social insects, are based on division of labour. Task differentiation among colony members is considered to be a major cause for the ecological success of eusocial insects (Wilson 1975; Chittka and Muller 2009). Aside from the fundamental divergence between reproductive and worker caste, individuals vary within one caste. In the majority of eusocial insect species, workers within colonies are monomorphic and can be separated into discrete task groups on the basis of their behaviour (behavioural castes) (Wilson 1976; Charbonneau and Dornhaus 2015). Task specialization of morphological worker castes (caste polyethism) is known in several ant species and in one species of bee so far (Grüter et al. 2012), but appears to constitute an exception to the rule.

Insect workers from the same colony can greatly differ in frequency, rate, sequence and ontogenetic timing of task performance (Jeanne 1991). Evidence exists that there are enormous differences in activity on different time scales among insect nestmates (O'Donnell and Jeanne 1992a; Hurd et al. 2003; Polidori et al. 2006; Tenczar et al. 2014; Perry et al. 2015). Some workers, often referred to as “elite” are incredibly active and productive (Hurd et al. 2003; Perry et al. 2015), while others are comparatively “lazy” (Charbonneau and Dornhaus 2015). Data available are generally limited by sample size and can represent only short periods of individuals' life. We are still far from understanding the causes and consequences of these huge inter-individual differences (Jeanne 1988; Ross and Matthews 1991; Dornhaus 2008; Chittka and Muller 2009).

Although in the majority of studies on division of labour “foraging” is regarded as a single task (Seeley 1982; Gordon 1996), alternative foraging activities (1) represent the contribution to different functional task at the colony level, (2) respond to different stimuli in the colony environment, possibly originating in spatially segregated parts of the nest, (3) can have differential costs for the foragers (O'Donnell and Jeanne 1992a; Biesmeijer and Tóth 1998), and (4) can

be partitioned, with task partitioning differences between and within species and colonies (Gordon, 1996; Matsuura & Yamane, 1990; Matsuura, 1991). In fact, division of labour is dynamic, can be restructured (e.g. according to colony size, see Jeanne 1986, Jeanson and Fewell 2007) and is constantly tuned to transient environmental conditions, through feedback mechanisms mediated by signals and cues within colonies (Nieh 2009; Johnson 2010; Santoro et al. 2015a).

Vespula yellowjackets (Vespinae) and the swarm-founding Polistinae (e.g. *Agelaia*, *Polybia*) show developed and populous wasp societies (Ross and Matthews 1991). Research focusing on the polyethism of *Vespula* spp. shows that, to a certain extent, worker tasks change in relation to individual age (temporal polyethism) (Potter 1964; Akre et al. 1976; Hurd et al. 2007; Kim et al. 2012), although these ontogenetic transitions do not seem to be as marked as, for example, in honey bees (Spradbery 1973; Hurd et al. 2007). In *Apis mellifera*, domestic duties such as nursing or ventilating are performed during the first days of life, guarding and foraging later (Seeley 1982). Vespulids have previously been thought to show similar patterns, but they do not switch as abruptly from one task to another, performing multiple tasks on short time-scales and showing great variability (Hurd et al. 2003; Hurd et al. 2007). Polyethic transitions in these wasps have been studied within foraging activity. During the first foraging days, the dominant activity is wood-pulp collection. Flesh collection tends to occupy the middle period of adult life. Throughout the foraging career, there is a progressive increase in fluid collection (Brian and Brian 1952; Potter 1964; Hurd et al. 2007; Kim et al. 2012). The onset of foraging appears to vary depending on the colony phase (Potter 1964).

Despite being considered monomorphic (unimodal distribution of sizes and absence of differential growth of body parts), *Vespula* workers from the same colony can greatly vary in their size. The average size of emerging adults and the variation among nestmates increase during colony development (Spradbery 1972). Within a colony, individuals observed foraging appear to be, on average, bigger than those staying in the nest (Spradbery 1973). According to

Herold (1952, in Spradbery 1973), one *V. germanica* worker is capable of carrying a load half of its weight (70-85 mg). Larger workers were reported to carry heavier loads and also to make more sorties per day than smaller nestmates. On the same species, Hurd et al. (2007) found that bigger workers tended to start their foraging activity earlier in their life. It was also noticed that workers of *V. sylvestris* were always slower at a particular foraging task than the bigger queens (Brian and Brian 1952). Taken together, these observations suggest a role of wasp workers' size in foraging patterns and performance, but empirical support for this hypothesis is weak.

For the colony, foraging is a fundamental requirement for its growth (producing workers) and to reach sexual maturity (producing males and gynes). For the individual worker, foraging is a dangerous and costly activity (O'Donnell and Jeanne 1992b; Biesmeijer and Tóth 1998). Leaving the nest shelter is a highly risk-taking decision and searching for resources in the field necessitates the consumption of energy and time (Wolf and Schmid-Hempel 1989; Robinson et al. 2009; Contrera et al. 2011). Social insect foragers are exposed to greater risk of predation and parasitization, and to greater thermal and physical stresses than their sedentary nestmates (Jeanne 1991). Moreover, foraging in flying insects implies high metabolic expenditure which might result in accelerated senescence and premature death (Neukirch 1982; Wolf and Schmid-Hempel 1989; Remolina et al. 2007). Hence, individual foraging enhances colony fitness at the expense of a forager's relative intra-colony fitness (Jeanne 1991).

With the present study, we studied the foraging behaviour of the common wasp *Vespula vulgaris*. We aimed to (i) Assess wasps' individuality, quantifying the intra-colonial variation in life-time foraging effort (investigating the presence of "elite" workers) and the degree of foraging task specialization among *V. vulgaris* nestmates, (ii) investigate the proximate causes of the variation and changes in foraging activity of adult *V. vulgaris* wasps (polyethism), testing how individual foraging patterns can be related to age and size (temporal and morphological polyethism). In particular, we tested the hypothesis that bigger workers are more

likely to forage, that they are more active foragers and more likely to perform specific foraging tasks. (iii) Finally, we studied the lifespan and survival of *V. vulgaris* workers and foragers in relation to their size (hypothesizing longer lifespan for bigger individuals) and their age when beginning the foraging activity (expecting that early foragers live less).

3.3 Materials and methods

3.3.1 Wasp colonies and study sites

The common wasp *V. vulgaris* (Hymenoptera: Vespidae) is an eusocial insect species that has become a notorious pest in New Zealand, likely originating from western Europe (Lester et al. 2014). Common wasps forage for wood-pulp, water, carbohydrates and proteins (from hunting or scavenging activities). The present study was conducted in New Zealand, on three colonies of *V. vulgaris* at different stages of ergonomic development. The nests were collected in the field and put in white pvc ventilated boxes. On the same day, they were carried in the designed research facility (2013: sleepout, Houghton Bay, Wellington; 2014: greenhouse, Plant & Food Research Limited, Lincoln; 2015: roof-top greenhouse, Victoria University of Wellington, Kelburn, Wellington – See below for further details) (Fig. 3.S1). The colonies were anesthetized with carbon dioxide, manipulated and relocated in study boxes, ventilated and provided with at least one transparent wall. The nest boxes were kept in darkness with cardboard boxes provided with ventilation holes. The wasps were allowed to freely forage in the field, in “natural conditions”, being forced to walk through a transparent perspex module when leaving and returning to the nest. The entrance module was designed on the base of Potter’s (1964) drawings, and shaped as a double funnel to direct the traffic and separate two lanes, one for the wasps going out (“outgoers”) and one for those returning from foraging trips (“incomers”) (Fig. 3.S2).

On 7/2/2013, one subterranean nest was collected and boxed in Wilton, Wellington (colony A). The nest was in early stage of colony development and small in size, having three combs (maximum diameter 14 cm), for a total of about 700 cells. The queen, 97 workers and 10 males were captured with the nest. Throughout the season, the envelope was rebuilt but the nest did not grow in size (Fig. 3.S1A).

On 20/1/2014, one subterranean colony was excavated in Christchurch, New Zealand (colony B). The nest was 40 cm underground, had almost no envelope and shared the exit with a bumblebee hive. We captured the queen and about 450 workers. The nest was of medium size, being composed by six combs (maximum diameter 17 cm), for a total of approximately 1500 cells. The nest box was placed in a greenhouse facility of Plant & Food Research Ltd. in Lincoln. Most of the site surrounding the greenhouse was covered in mown grass, with several tree patches dominated by *Quercus* spp. The nest grew in size during the season, producing several hundred workers, and males started emerging at the end of February. After the death of the last tagged workers, in the middle of March, the nest was opened and examined. The envelope completely filled the nest box, the combs were expanded (7000 cm³), and queen cells (about 300, uncapped) were built, for a total of about 5400 cells (Fig. 3.S1B).

On 24/2/2015, one aerial nest was collected in the Stokes Valley, New Zealand (colony C). The nest was of big size, being composed by 8 combs (maximum diameter 23 cm) for a total of total of approximately 6000 cells. The nest was enclosed in a transparent plastic box (45*30*30 cm) and transported on the rooftop of the New Kirk Building, Victoria University of Wellington, Kelburn. The box was manipulated and set up in a greenhouse room, kept at 28°, on 3/3/2015. On the first days, we observed wasps to repair the damaged parts of the envelope. The nest did not grow in size during the following weeks. Unfortunately, one month after the initiation of the study, on 24/3/2014, one worker was filmed at the nest entrance while carrying the corpse of the dead queen. On the following days, the colony foraging activity dropped but we kept recording

the workers provided with RFID tags (last record from 11/4/2014) and filming the nest entrance until 15/4/2014, when workers' activity ceased and the nest was almost deserted (Fig. 3.S1C).

3.3.2 Obtaining individually marked wasp workers of known age and size

In 2013, all the 97 adult workers present in colony A were tagged with RFID tags (microsensys GmbH, mic3®-TAG 16k; dimension: 1.9×1.6×0.5 mm; weight: 5 mg) within 24 h from nest collection. The tags were secured to the thorax tergite with glue (Quick Fix® Supa Glue, Selleys), after lightly anesthetizing the wasps with carbon dioxide. Between 17/2/2013 and 17/3/2013, during daily observations (1h minimum), additional 83 untagged foragers were captured at the entrance and provided with RFID tags ("workers An"). On 15/3/2013, a second nest was excavated in Houghton Bay, Wellington, and its combs used as a known-age workers' reservoir. A total 120 workers emerging from these combs were tagged with RFID tags and introduced into colony A from 16/3/2013 to 18/3/2013 ("workers Ap") (Fig. 3.S3) (Hurd et al. 2007).

To obtain known-age workers in 2014 and 2015, we kept aside capped brood combs during nests' collection from the field sites, which were held in an incubator (30°C, 50% + relative humidity, and complete darkness). RFID-tagged wasps were additionally coloured on the first abdominal tergite, according to the day of emergence (Sharpie® Water-Based Paint Markers). For colony B, we incubated two combs. Between 21/1/2014 and 31/1/2014, a total of 300 adults emerging were provided with RFID tags and introduced in the colony of origin within 24 hours from emergence. Additional 130 known age workers (between 1/2/2014 and 6/2/2014) and 200 foragers, captured at the nest entrance between 29/1/2014 and 11/2/2014, were individually marked with plastic numbered tags (queen numbering kit, Ecrotek Ltd; weight: 2mg) and introduced in the colony. For colony C, 4 main pieces of combs were extracted from the nest.

From 4/3/2015 to 18/3/2015, we daily collected, tagged, measured and inserted in the natal nest emerging workers. A total of 644 known age individuals were provided with RFID tags (Fig. 3.S3).

As a proxy for body size of the wasps, we measured the head width of all the known-age workers and marked workers from colony B and C, using a digital calliper to the nearest 0.01 mm (Polidori et al. 2011) (Fig. 3.S3B, 3.S4).

3.3.3 RFID data

The activity of the incomers and outgoers provided with RFID tags was automatically monitored from the time of their introduction inside the nest, corresponding to their first day of life. Whenever a tagged wasp walked by the nest entrance module, its identity and the time to the milliseconds were recorded by one RFID system (microsensys GmbH, iID@HOSTtypeMAYA4.1). In 2013, the host was connected to two RFID tunnel scanners (iID@MAYAreadermodule4.1), each one fitting one entrance lane. In 2014 and 2015, two RFID scanners of the same type were placed on each lane, to have more precise information on the direction taken by the individuals inside the entrance (Fig. 3.S2). The scanners were placed 5 cm from each other, to avoid interference among them. To avoid confusing effects from circling (walking in circle in the entrance module), orientating (performing orientation flights when briefly exiting the nest for the first times), drifting (joining other colonies) and overnighing (spending the night outside of the nest) workers, we considered “foraging trips” periods of time outside of the nest longer than 2 minutes and shorter than 8 hours (personal observations showed that 2 minutes was the minimum time necessary for a wasp to walk through the entrance both ways and take a resource in front of the nest entrance; the time window of 8 hours roughly corresponded to the night-time foraging stop of the wasp colonies). Workers were considered “foragers” if they performed at least one trip longer than 2 minutes. It was possible to estimate wasps’ lifespan (day of emergence to day of last record) a variety of foraging

activity measures, including (1) foraging onset (age at first foraging trip) (2) foraging tenure (days from the first to the last trip) (3) number of lifetime trips (4) number of trips per foraging day (5) total time spent out of the nest and in the field (including intervals shorter than 2 minutes and longer than 8 hours) (6) each trip length.

3.3.4 Observational data

In 2014 and 2015, we collected longitudinal data on the foraging task performed by the individually marked wasps and their nestmates. Colony B nest entrance was filmed using a Sony Handycam Dcr Sr40 Hdd 30gb and observed from 28/1/2014 to 17/3/2014, for a total of 145 hours of colony activity. Colony C nest entrance was continuously filmed from 14/03/2015 to 11/04/2015, using a raspberry picam videocamera (<https://www.raspberrypi.org/help/camera-module-setup/>) (Fig. 3.S2). For 1 hour focal periods, loads carried by the tagged wasps were observed and recorded. Each focal period was immediately preceded or followed by a 10 minute interval during which loads of all unmarked individuals were recorded. During the focal observations, whenever a wasp with RFID tag was passing by the first of the two readers in the incomers' lane, the resource item and time to the nearest second were recorded. We distinguished five behavioural categories (1) empty (2) with fluid, partially loaded (3) with fluid, fully loaded (4) with pulp (5) with a flesh load (Fig. 3.1, Plate I, page 152). Some foragers had mixed fluid and solid (flesh or pulp) loads. In case of mixed loads, priority for categorization was given to the solid load, since the associated fluid load would likely come from the same source (Crop liquid of flesh carriers being probably extracted from prey during malaxation) (Archer 1977), or be functional to the same task (crop fluid being used by pulp foragers to moisten wood fibres) (Archer 2012). Observational load records for wasps with RFID tags were subsequently matched manually with traffic data from the RFID reader, so that each load type carried by a wasp provided with RFID tag could be attributed to an individual, on the basis of the timestamp and wasp's abdomen colour.

3.3.5 Individuality (elitism and specialization)

We first quantified the proportion of RFID-tagged individuals becoming foragers within each of the three colonies studied. Then, among foragers within each colony, we assessed the variation in the foraging activity parameters measured. We quantified the degree of foraging effort variation, elitism, and foraging task specialization. To estimate the individual foraging effort, we looked at the average number of trips per foraging day for all the individuals provided with RFID tag in colonies A, B, C. The number of trips per foraging day was used to quantify individual variation in foraging effort, using Roughgarden's index ($R's I$) (Roughgarden 1974) for continuous data (Bolnick et al. 2002). This index is based on the within-individual component and the between-individual component of the total niche width ($TNW = WIC + BIC$) and equals WIC/TNW . $R's I$ was calculated using Indspec1.exe (Bolnick et al. 2002), treating the number of individual trips per each foraging day as one value. The measure obtained was used to quantify the degree of elitism, the variation in the workers' foraging effort and its consistence over their foraging tenure.

To measure individual task specialization and the variation in foraging careers, we considered all individually marked workers from colonies B and C observed with loads, using the five behavioural categories described above. The overlap between the foraging tasks performed during life by each individual i (individual foraging careers) and the colony task allocation, obtained considering all the loads recorded within each colony, was measured with the PS index adapted to individual-level analysis (PSi , Bolnick et al. 2002). The mean value of PSi (IS) expresses the average individual specialization for the colony. IS was calculated using Indspec1.exe (Bolnick et al. 2002), treating each type of behavioural category for foraging wasp workers as a food item category and considering the colony as one population. Both $R's I$ and IS values range from 0 to 1. The lower the value, the less overlap among individuals and hence the higher

the degree of variation (stronger elitism and specialization) between individuals and within each colony.

3.3.6 Polyethism

For colonies B and C, using the films, we were able to investigate foragers' polyethism (variation in foraging careers and change in foraging tasks performed) in relation their age and size. To detect age-related behavioural changes (temporal polyethism), we first measured the number of foraging trips per day in relation to individual age for all the known age workers provided with RFID tags. Then, for the all the known-age workers observed with loads, we calculated two values, the first performance of a task (FPT) (Naug and Gadagkar 1998) and the probability of task performance (PTP) (Seeley 1982). FPT represents the average day of first observed performance of a certain foraging task by known age workers. PTP is the probability that a given forager will perform a certain task at a certain age, and is calculated as:

$$p_{ij} = \frac{n_{ij}}{\sum_{i=1}^k n_{ij}}$$

Where p_{ij} is the probability to perform the foraging task i by a member of age group j , n_{ij} is the number of performances of task i observed for members of age group j , k is the total number of behavioural categories.

To investigate the influence of size in the foraging activities (morphological polyethism sensu lato, Torres et al. 2013), we used colony B and C. To provide an index of the degree of size variation in these colonies, we calculated a coefficient of variation (CV = head width standard deviation/mean head width). We tested whether bigger workers (i) are more likely to become foragers, (ii) are more active foragers (iii) are more likely to perform particular foraging tasks. (i) We first compared the size of known-age individuals from colony B with that of the foragers captured and measured from the same colony during the activity of the same known-age workers. Then, on the basis of their different size distributions, we grouped the known age workers provided with RFID tags

in three relative size categories (2014, colony B: small, head width < 3.48 mm; medium, head width between 3.48 and 3.52 mm; big, head width > 3.52 mm; 2015, colony C: small, head width < 3.50 mm; medium, head width between 3.50 and 3.60 mm; big, head width > 3.60 mm) and tested for differences in the progression of the workers from the three groups becoming foragers. (ii) Among the known-age workers provided with RFID tags that foraged (performed at least on trip longer than 2 minutes), we tested for a correlation between individual size and foraging activity measures (foraging onset, foraging tenure, lifetime trips, average number of trips per foraging day, total time out, average trip length). (iii) For each forager observed with at least 5 loads, the relative “fixation” for each foraging task was quantified using standardized residuals (observed-expected)/expected^{1/2}). The expected values were equivalent to the frequency of each foraging task at the level of the whole workforce. On the basis of the highest standardized residual value obtained, each worker was assigned to the corresponding foraging task group. To test whether individual size has an effect on the performance of alternative foraging tasks, we tested for differences in average size of workers among foraging groups. Given sample sizes (few individual foraging trips and almost no pulp foraging in colony C), only colony B was used.

3.3.7 Survival in relation to size and foraging onset

We looked at the survival of the known age wasps provided with RFID tags (colonies B and C). For each colony, we focused on the survival in relation to body size of (i) all the workers, (ii) all the foragers (iii) all the foragers pooled in the three size groups above described. We also investigated the survival of foragers in relation to their foraging onset, pooling the individuals in three foraging onset groups, equivalent for both colony B and colony C (early onset: before 5 days old; average onset: between 5 and 10 days old; late onset: older than 10 days).

3.3.8 Data filtering and analysis

RFID data handling and filtering was performed with Track-a-Forager1.0.exe (Van Geystelen, personal communication). The program (1) bundles the scans in the raw RFID data by tag and scanner so that rapid-succession scans are treated as one scanning event within a certain timespan, (2) determines the events of entering (IN) or exiting (OUT) the nest, based on the bundles generated in the previous step, (3) the IN, OUT, events are used to determine the foraging trips and their lengths. User-defined time constraints can be applied on all three steps (present study cut-off settings: cluster = 20 sec, in/out = 20 sec, flight minimal = 10 sec, flight maximal = unlimited time). The Track-a-Forager output was subsequently filtered not considering foraging trips shorter than 2 minutes and longer than 8 hours (see above).

The indices of overlap to measure the intra-colonial degree of elitism and specialization (R's I and IS) were calculated with the IndSpec1.exe (Bolnick et al. 2002). IndSpec uses a nonparametric Monte Carlo procedure to generate replicate null diet matrices drawn from the population distribution, from which P values can be computed (Bolnick et al. 2002). Ten thousand replicates in Monte Carlo bootstrap simulations were performed.

The following statistical analyses were performed using IBM SPSS Statistics 21 (IBM Corp. 2012) and R 3.1.0 (R Development Core Team 2013). To test for differences in the proportion of foragers starting foraging among colonies, we performed likelihood ratio Chi-Square test. To test for relationships between foraging activity measures (number of life-time trips and number of trips per foraging day) and for an effect of size on the foraging activity measures, linear models were used (Pearson's r). To analyse the association between individual age and the average number of trips, we used linear and quadratic regression models. To analyse the association between individual age and PTP, linear and curvilinear regression models were used. To test for differences in FPT among alternative foraging tasks (half fluid, full fluid, pulp, flesh) and to compare the size of known age workers introduced in the nest and foragers from the

colony B, one-way ANOVA was used. The subsequent multiple comparisons were performed using the Tukey-Kramer post hoc test. To test whether the individuals in each foraging group differed in size, we performed non-parametric Kruskal-Wallis test for an overall comparison among foraging groups, and subsequently a Mann-Whitney test in two by two comparisons between groups. A Kaplan-Meier survival analysis was performed to investigate the survival of (i) all workers and all foragers in relation to their size, (ii) foragers pooled in size groups, (iii) foragers pooled in foraging onset groups, and (iv) to test for differences in the proportion of workers getting to forage within size groups. After verifying the non-violation of the proportional hazard assumption, log rank (Mantel-Cox) and Breslow (Generalized Wilcoxon) statistics were used to compare the survival curves. When colonies B and C were showing different or opposite trends, they were considered separately. When colonies showed the same significant trends, data were pooled. In the following text, mean values are reported \pm standard deviation.

3.4 Results

Vespula vulgaris nestmates showed enormous variability in foraging behaviour over their entire lives, and we found evidence of both elitism and individual specialization (3.4.1). The variation observed among wasp foragers was only partially explained by individual age (3.4.2) and size (3.4.3). We found that common wasp workers can live up to 7 weeks, longer than previously thought. Individuals that started foraging earlier died earlier (3.4.4).

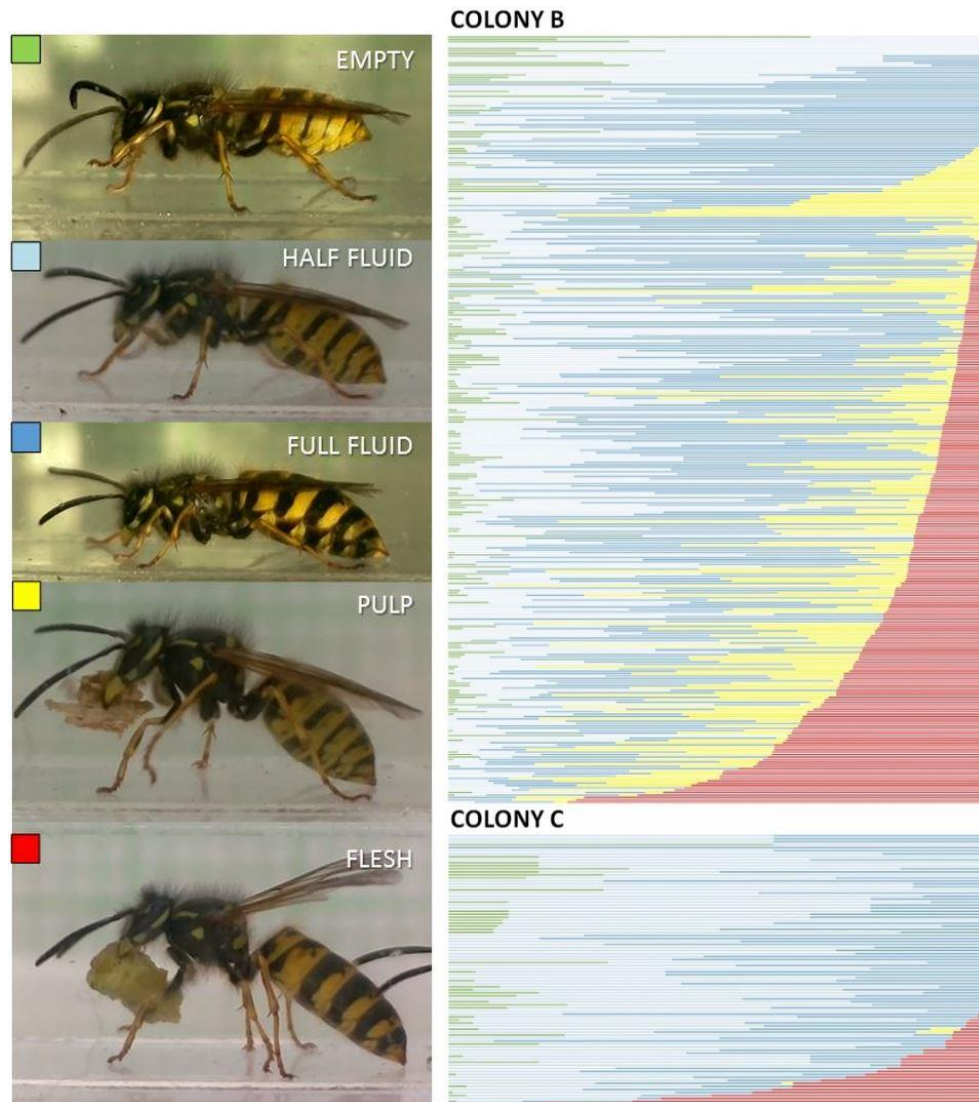


Fig. 3.1 Common wasp foragers. (left) Behavioural categories distinguished (foraging tasks): empty; with fluid, partially loaded; with fluid, fully loaded; with a wooden pulp load; with a flesh load (See also Plate I, page 152). (right) Inter-individual, life-time variation in the foraging tasks performed among nestmates from colonies B (2014) and C (2015). Each horizontal line represents one individually marked wasp worker and its relative contribution over life to the alternative foraging tasks (load types ratio). Only individuals observed at least five times are represented.

3.4.1 Individuality (elitism and specialization)

The proportion of workers provided with RFID tag becoming foragers (performing at least one trip longer than 2 minutes) varied between colonies ($G^2 = 201.99$, $df = 2$, $p < 0.001$) and decreased with increasing colony size (Fig. 3.2).

Colony B was the only one that could be considered representative of a “successful” colony, as it grew during the season and got to the construction of queen cells. The overall activity window for the workers from colonies A, B, and C was respectively 13/2/2013-11/4/2013, 24/1/2014-15/3/2014, and 6/3/2015-11/4/2015. Over the three years, among those individuals that foraged ($n = 607$), we found extreme variability in every foraging activity parameter measured (Table 3.1). The length of foraging tenure was 9.4 ± 9.9 days, lasting from 1 to 43 days. On average, the total number of individual foraging trips was 88.8 ± 138.1 and varied from 1 to 819. Overall, the average number of individual trips per foraging day was 6.1 ± 7.2 and varied from 1 to 53 (Fig. 3.2). For workers of colonies B and C, there was a strong linear relationship between the average number of trips per foraging day and the number of lifetime trips ($r = 0.832$, $n = 401$, $p < 0.001$). In the three colonies, the foraging trip length varied enormously (Table 3.1). Over their lifetime, 38% of the foragers spent at least once 8 or more consecutive hours in the field (overnight) and 12% of the wasps more than one day, and up to 19 days, away from their colony (likely drifting, i.e. going into other wasp colonies).

Table 3.1 Foraging activity measures for the three wasp colonies studied, including intra-colonial diversity indices (mean \pm SD). The total number of lifetime trips and time out for nest workers from colony A represent under-estimates. P-values for individual variation in daily foraging effort (measured by Roughgarden’s index, R’s I – N values for Colony A Nest: 1272; A Foreign: 386; B: 3071; C: 699) and foraging task specialization (measured by proportional similarity index IS) were obtained from ten thousand replicates in Monte Carlo bootstrap simulations. * $p = 0.016$; ** $p = 0.008$; *** $p < 0.001$.

COLONY	A		B	C
Workers	Nest	Foreign		
Foraging onset (age in days)	na	na	6.8 ± 2.9	7.4 ± 2.8
Foraging tenure (days)	8.7 ± 8.1	6.4 ± 5.5	16.5 ± 11.5	3.5 ± 3.9
N lifetime trips	(112.8 ± 157.2)	30.4 ± 43.6	158.1 ± 161.9	17.7 ± 38.6
N trips / foraging day	9.4 ± 9.4	3.6 ± 4.7	7.8 ± 7.4	2.8 ± 2.8
Total time out (hours)	(86 ± 521)	87 ± 352	147 ± 1107	40 ± 304
Average trip length (min)	30 ± 41	61 ± 72	25 ± 25	181 ± 454
R’s I (ELITISM)	0.461 ***	0.482 *	0.563 ***	0.365 **
IS (SPECIALIZATION)	na	na	0.668 ***	0.688 ***

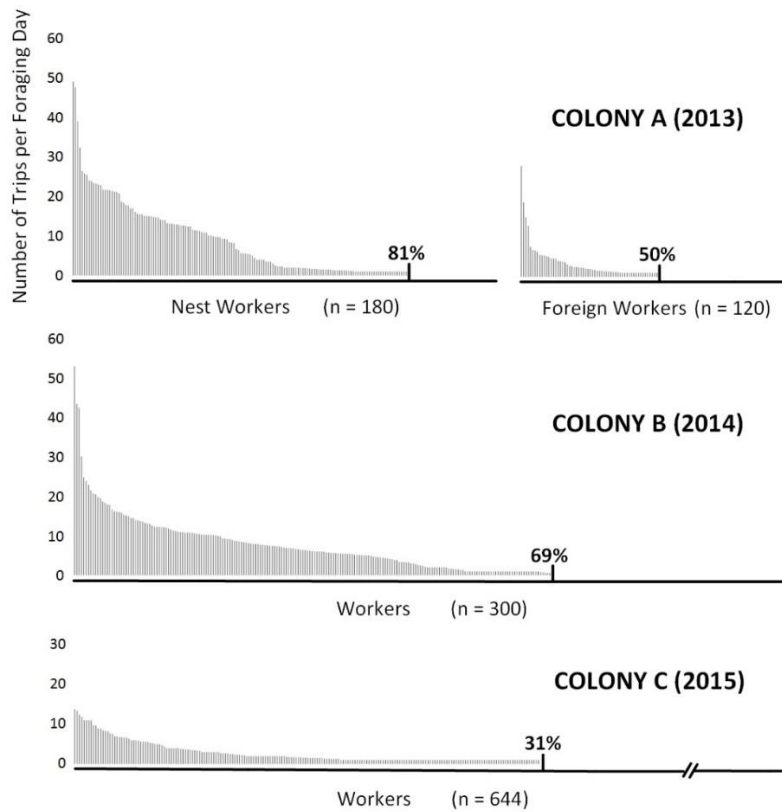


Fig. 3.2 Proportion of individuals provided with RFID tags foraging (the thick horizontal black line represents the sample size per group of workers) and individual variation in foraging effort, measured as the average number of trips per foraging day (each thin vertical line represents the lifetime average for one individual).

We found strong and significant intra-colonial variation in (i) foraging effort (Table 3.1, Fig. 3.2) and (ii) foraging task specialization (Table 3.1, Fig. 3.1). We found evidence of elitism, measuring statistically significant differences in foraging effort within the three colonies. Despite more overlap (higher R's I values), differences were particularly significant among nest workers from colony A and within colony B workforce, due to increased sample size (Table 3.1 caption). Workers from the smallest colony (A) performed the highest number of trip per foraging day (Table 3.1). A total of 324 individually marked foragers from colony B and 90 from colony C were observed returning to the nest with 5 or more loads (Fig. 3.1). Individuals varied greatly in the foraging tasks performed throughout their life, specializing in alternative tasks. Highly significant

foraging task specialization was measured within colonies' B and C workforce (IS index, Table 3.1).

3.4.2 Polyethism by age

Despite the extreme inter-individual variability, we found that there was a significant relationship between worker's age and the average number of trips performed, first increasing and then decreasing over lifetime (Fig. 3.3). The relationship was best described by a quadratic function both in colony B (linear: $R^2_{\text{adj}} = 0.063$, $F_{1,45} = 3.01$, $p = 0.090$; quadratic $R^2_{\text{adj}} = 0.870$, $F_{2,44} = 147.12$, $p < 0.001$) and colony C (linear: $R^2_{\text{adj}} = 0.162$, $F_{1,28} = 5.39$, $p = 0.028$; quadratic $R^2_{\text{adj}} = 0.603$, $F_{2,27} = 20.52$, $p < 0.001$). The average number of trips per foraging day increased for most of the workers' life in both colonies B and C (Fig. 3.3).

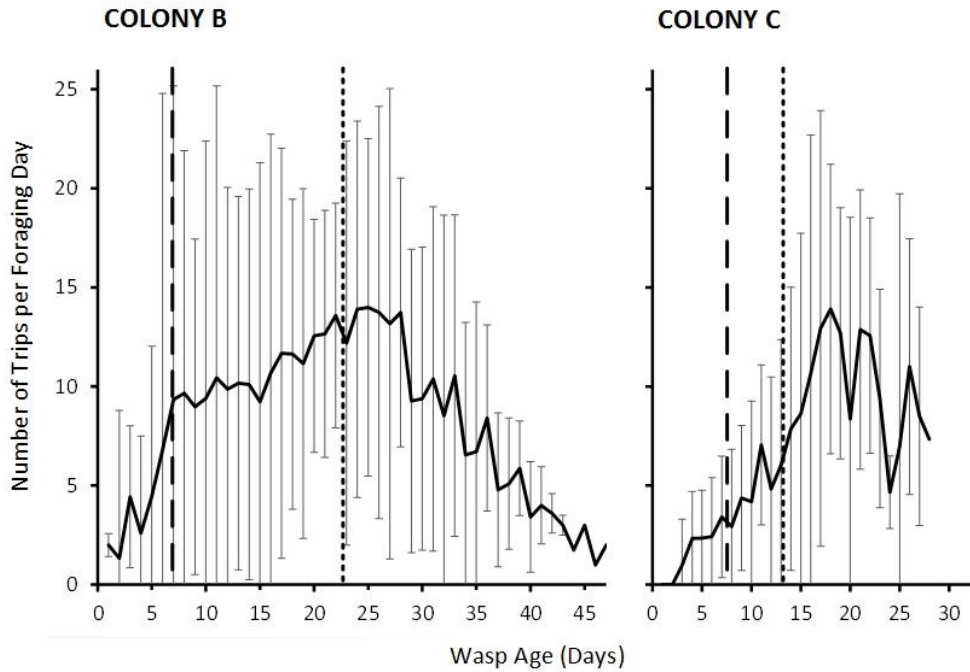


Fig. 3.3 Number of foraging trips per day in relation to *Vespula vulgaris* worker age. The bold line represents the average, error bars the standard deviation. For each colony, the vertical dashed line represent the average age when foraging commenced, the vertical dotted line the average individual age when the last trip was performed (cfr. Fig. 1.6).

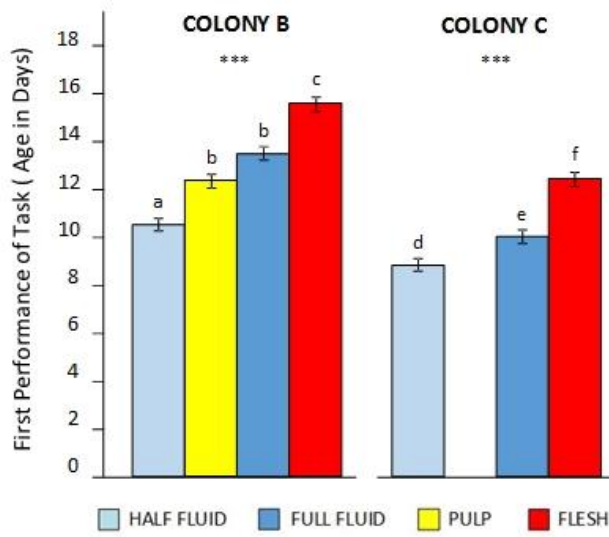


Fig. 3.4 Foraging polyethism by age in *Vespula vulgaris*. Days of age at first performance of task (FPT) for the alternative foraging tasks (mean \pm SE). Asterisks indicate significant differences in FPT (***) = $p < 0.001$ by one-way ANOVA). Considering each colony separately, significant differences within task groups are indicated with different letters ($P < 0.01$ by Tukey-Kramer test). Refer to Fig. 3.S1 for additional details.

Comparing the first performance for each foraging task, we found that there was a significant effect of worker's age on the FPT for the two colonies (one-way ANOVA: for colony B, $F_{3,853} = 50.737$, $p < 0.001$; for colony C, $F_{2,281} = 18.725$, $p < 0.001$) (Fig. 3.4). Multiple comparison analysis revealed that in both colonies B and C, the alternative foraging tasks were performed for the first time at different ages, and in the following order: half fluid, pulp and full fluid, finally flesh carrier (Tukey-Kramer test: between all task groups for the 2014 colony, $p < 0.001$, except between pulp and full fluid, $P = 0.062$; for the 2015 colony, for all task groups, $p < 0.01$) (Fig. 3.4).

Analysing the PTP for each load type, we found that curvilinear models explained better the association between individual age and PTP (Fig. 3.5). For incoming foragers, the probability of not having any load (PTP for empty carriers) decreased with age (linear: $R^2_{\text{adj}} = 0.044$, $F_{1,3037} = 139.44$, $p < 0.001$; curvilinear $R^2_{\text{adj}} = 0.936$, $F_{2,3036} = 22314.22$, $p < 0.001$). The probability to have half full crops (PTP for half fluid carriers) decreased with age (linear: $R^2_{\text{adj}} = 0.020$, $F_{1,3037} = 63.22$, $p < 0.0001$; curvilinear $R^2_{\text{adj}} = 0.937$, $F_{2,3036} = 22459.54$, $p < 0.001$). The probability to have crops full of liquid (PTP for full fluid carriers) increased with age (linear: $R^2_{\text{adj}} = 0.106$, $F_{1,3037} = 361.15$, $p < 0.0001$; curvilinear $R^2_{\text{adj}} = 0.942$, $F_{2,3036} = 24778.64$, $p < 0.001$). The probability to forage for pulp first

3. The Common Wasp Radio-Tagged

increased and then decreased after 14 days of age (linear: $R^2_{\text{adj}} = 0.045$, $F_{1, 3037} = 145.17$, $p < 0.001$; curvilinear $R^2_{\text{adj}} = 0.975$, $F_{2, 3036} = 29492.03$, $p < 0.001$). The probability to return from a foraging trip with flesh increased with age (linear: $R^2_{\text{adj}} = 0.121$, $F_{1, 3037} = 45.13$, $p < 0.001$; curvilinear $R^2_{\text{adj}} = 0.959$, $F_{2, 3036} = 17217.55$, $p < 0.001$) (Fig. 3.5).

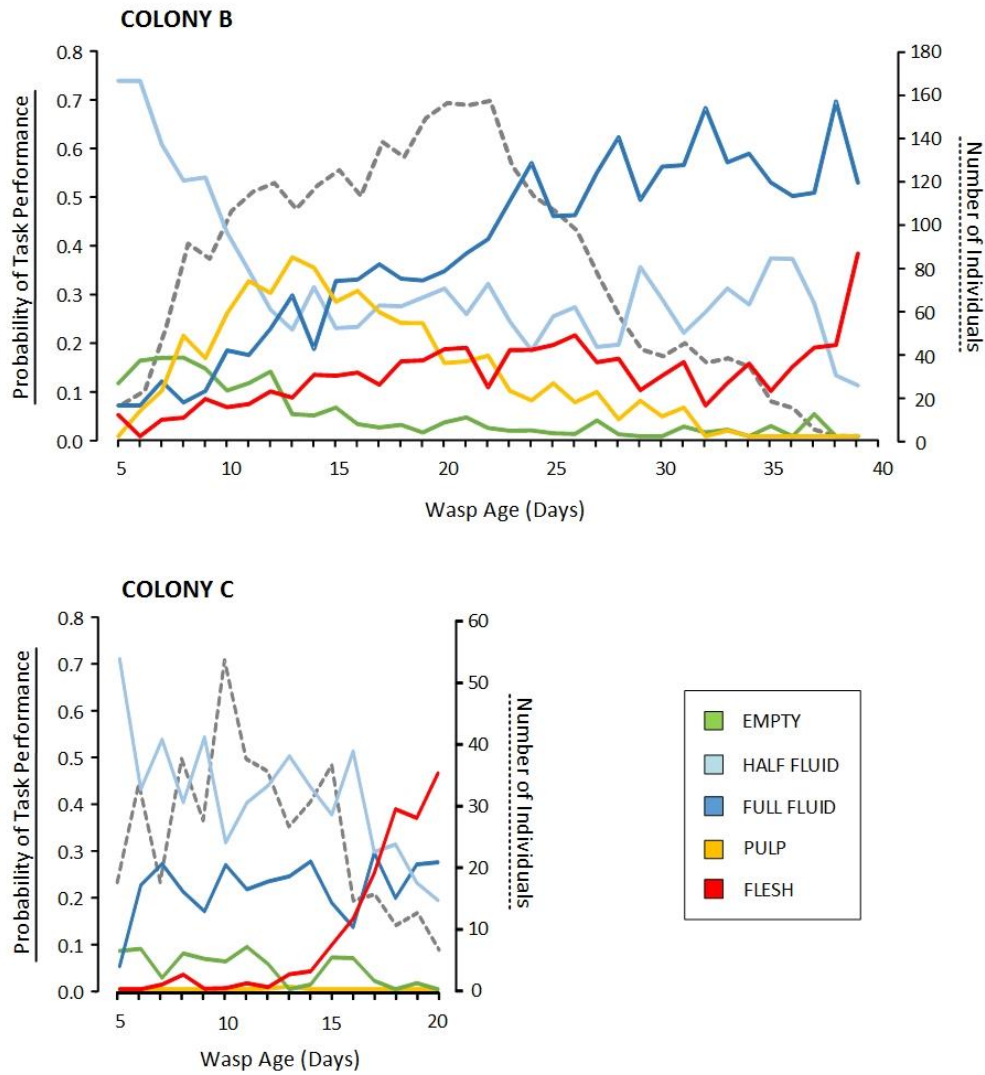


Fig. 3.5 Foraging polyethism by age in *Vespa vulgaris*. Probability of task performance (PTP) for each foraging task (in the text box), as a function of wasp age. The probability of not carrying any load (empty) is included. The number of individuals per each age group is represented by the grey dashed line (cfr. Fig. 1.5).

Table 3.2 Correlation coefficient (Pearson's r with Bonferroni correction) between size of *Vespula vulgaris* foraging workers provided with RFID tags (measured as individuals' maximum head width) and measures of foraging activity. Significant relationships are in bold. $** = p < 0.01$.

Individual Size and Foraging Activity	r	p	n
COLONY B			202
Foraging onset	-0.217	0.002	**
Foraging tenure	-0.024	0.739	
N lifetime trips	0.085	0.228	
N trips / foraging day	0.059	0.409	
Total time out	0.008	0.915	
Average trip length	-0.135	0.055	
COLONY C			198
Foraging onset	0.101	0.157	
Foraging tenure	0.104	0.144	
N lifetime trips	0.104	0.146	
N trips / foraging day	0.129	0.071	
Total time out	0.132	0.064	
Average trip length	-0.04	0.579	

3.4.3 Polyethism by size

There was great variability in workers' head width, ranging from 2.79 to 3.70 mm. Overall, as expected given the difference in the colony size, workers from the bigger colony C were on average bigger and more variable in size than workers from colony B (colony B head width: 3.51 ± 0.08 , 3.07 to 3.69; CV=0.02. Colony C: 3.54 ± 0.09 , 2.79 to 3.70; CV=0.03) (Fig. 3.6). We found that the foragers captured from colony B (head width: 3.57 ± 0.06) were on average significantly bigger than the known age workers introduced into the colony (head width: 3.48 ± 0.07) ($F_{1,581} = 175.72$, $p < 0.0001$). Bigger workers from colony B started foraging earlier (Table 3.2). In the two by two comparisons between relative size classes, a significantly lower proportion of small workers from colony B got to forage compared to medium-sized (log rank: $\chi^2_1 = 2.75$, $p = 0.097$; Breslow: $\chi^2_1 = 6.84$, $p = 0.009$) and big-sized workers (log rank: $\chi^2_1 = 5.38$, $p = 0.020$; Breslow: $\chi^2_1 = 11.52$, $p = 0.001$) (Fig. 3.7). Although no other significant relationships were found between size of the workers and foraging

activity measures, relatively large workers seemed to be more active foragers, tending to perform shorter trips in colony B while performing more trips per day and spending more time out in colony C ($p < 0.10$; Table 3.2). In colony C, size had no significant effect on the onset of foraging (Table 3.2) nor on the proportion of workers foraging within size classes in colony C (Fig. 3.7). Workers grouped by foraging task showed significant differences in size, those returning disproportionately more often with no loads being relatively smaller and those returning with flesh loads being relatively larger (Kruskal-Wallis, $\chi^2_4 = 10.88$, $p = 0.028$) (Fig. 3.6).

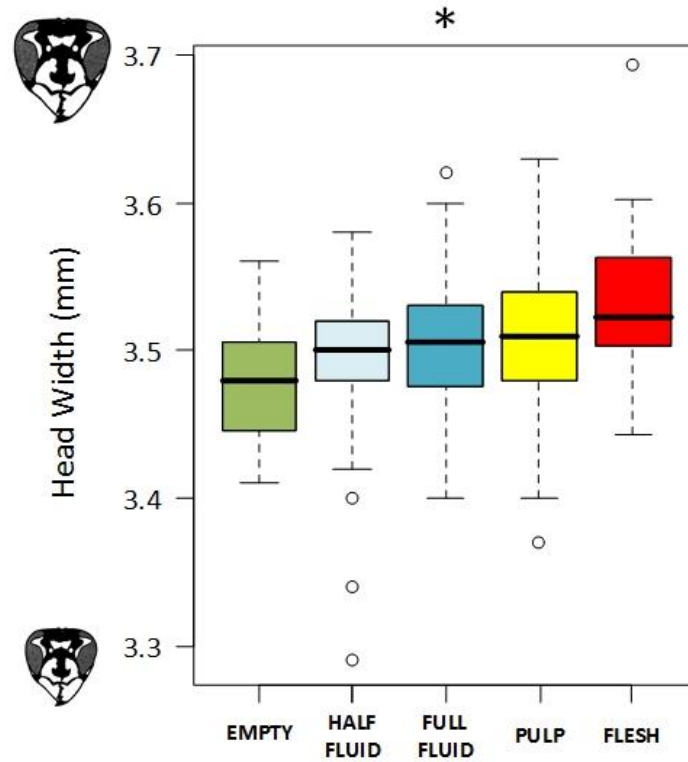


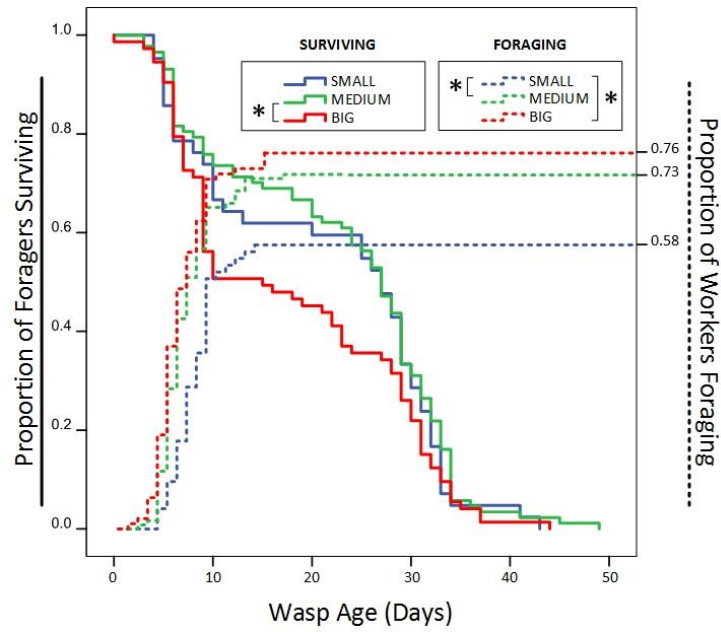
Fig. 3.6 Foraging polyethism by size (head width in mm) in *Vespula vulgaris*. Differences in size among workers fixated on alternative foraging tasks (colony B). Each forager observed with at least 5 loads was assigned to one foraging group (number of individuals in parenthesis) on the basis of the highest standard residual value obtained, corresponding to the foraging task that was performed more frequently compared to the workforce average (overall $* = p < 0.05$ by Kruskal-Wallis test). Significant differences obtained in two by two comparisons between foraging groups are indicated with different letters ($a-b$, $b-c$ $p < 0.05$; $a-d$ $p < 0.01$ by Mann-Whitney U test).

3.4.4 Survival

The lifespan of *V. vulgaris* workers varied from 1 to 50 days of life (one individual with numbered tag) (RFID data, colony B: 18.3 ± 12.3 days; colony C: 9.2 ± 4.8 days). Foragers lived on average longer than nestmates never getting to forage (colony B: 28.3 ± 8.8 days; colony C: 12.7 ± 5.1). In colony B, considering all the RFID-tagged workers (foraging or not) we found that individual size did not predict survival ($\chi^2_{33} = 41.55, p = 0.146$). Considering only foragers, there was a negative effect of size on survival ($\chi^2_{28} = 44.56, p = 0.024$). Considering the foragers grouped in the three size classes and performing two by two comparisons, differences in survival were found between medium and big-sized wasps, with larger foragers dying earlier ($\chi^2_1 = 4.10, p = 0.043$). Big foragers were less likely to survive earlier in life, half of them being dead by the 10th day of life (Fig. 3.7, colony B). Pooling foragers into three foraging onset groups (early, average, late onset) we found that workers that started foraging later survived longer ($\chi^2_2 = 6.92, p = 0.031$).

In colony C, considering all the RFID-tagged workers (foraging or not) we found that individual size did not predict survival ($\chi^2_{44} = 54.89, p = 0.126$). Considering only foragers, there was a strong positive effect of size on survival ($\chi^2_{37} = 108.78, p < 0.001$). Considering the foragers grouped in the three size classes and performing two by two comparisons, we found that big-sized foragers tended to live longer than small-sized foragers ($\chi^2_1 = 4.88, p = 0.027$), (Fig. 3.7) and medium-sized workers ($\chi^2_1 = 4.09, p = 0.043$). Big foragers tended to show increased survival probability later in life (Fig. 3.7, colony C). Pooling foragers into the three foraging onset groups (early, average, late onset) we found that workers starting foraging later survived longer (colony B: log rank: $\chi^2_2 = 6.92, p = 0.031$; Breslow: $\chi^2_2 = 9.41, p < 0.01$; colony C log rank: $\chi^2_2 = 14.98, p = 0.001$; Breslow: $\chi^2_2 = 42.37, p < 0.001$) (see Fig. 3.8 for data from colonies B and C pooled).

COLONY B



COLONY C

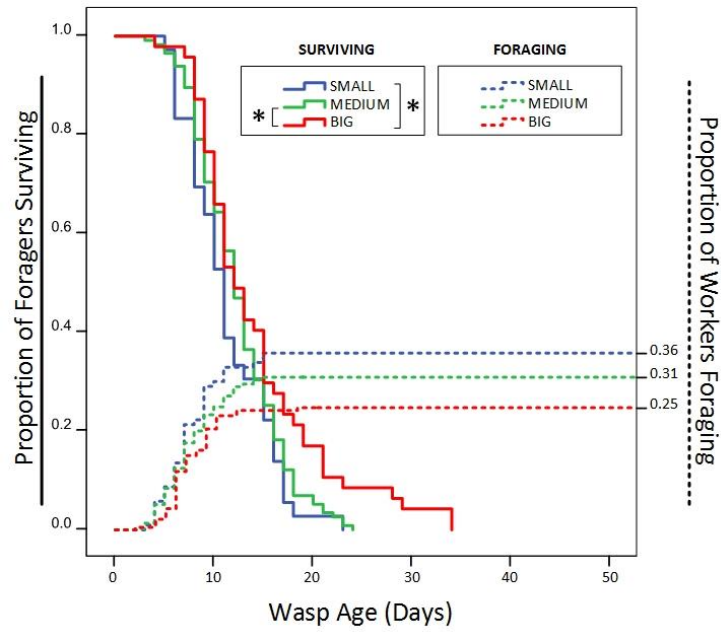


Fig. 3.7 Proportion of wasp workers foraging at a given age (dashed lines) and survival curves for foragers (continuous lines) for the three relative size groups in colony B and colony C. Significant differences between groups ($p < 0.05$) detected alternatively by Breslow (colony B) or Log Rank (colony C) statistics in two by two comparisons are marked with asterisks (See main text for details).

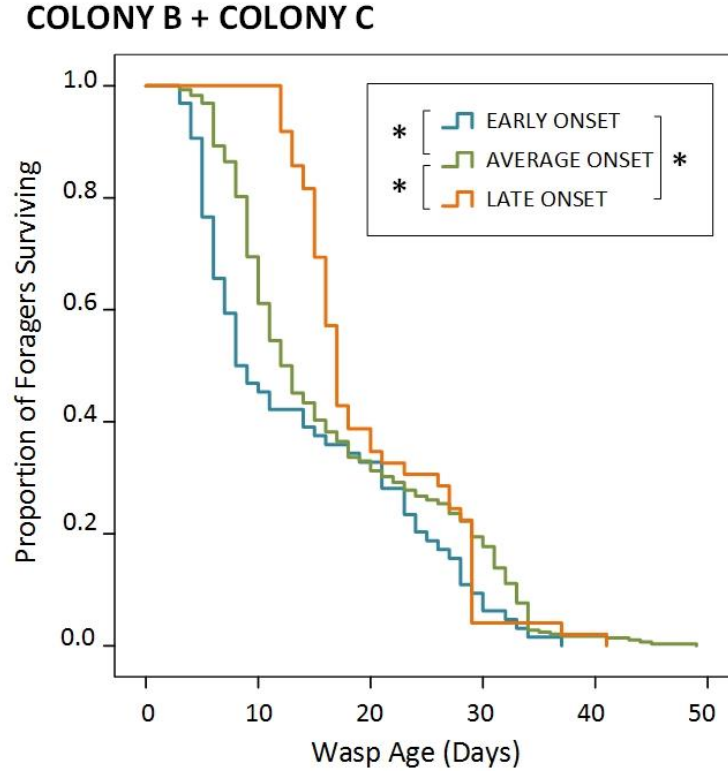


Fig. 3.8 Survival curves for foragers of colony B and C pooled in the three foraging onset groups (early onset: first foraging trip between 1 and 5 days old. Overall, workers starting foraging earlier survived for a shorter time (log rank: $\chi^2_2 = 8.99$, $p = 0.011$; Breslow: $\chi^2_2 = 23.56$, $p < 0.001$). Significant differences between groups ($p < 0.05$) detected alternatively by Breslow or Log Rank statistics in two by two comparisons are marked with asterisks (See main text for statistics within the two colonies).

3.5 Discussion

This study is one of few quantifying the behavioural variability within eusocial insect workforces. For the first time in a wasp species, we monitored the lifetime activity patterns of a large number of workers using RFID technology.

3.5.1 Individuality (elitism and specialization)

Most of the previous studies focusing on the division of labour of social insect workers have described “average” phenotypes and concentrated attention on associated mechanisms such as temporal polyethism, explaining the inter-

individual variation observed at any one time as the result of overlapping generations and different age-related behavioural repertoires (Calderone and Page 1988; Ross and Matthews 1991; Jeanson and Weidenmüller 2013). Yet, dramatic behavioural differences can be found among apparently identical insect workers of the same cohort and age (Ravary et al. 2007). Thanks to new technologies such as RFID systems, we now know that these differences can be enormous and consistent in time (Tenczar et al. 2014; Perry et al. 2015). The study of the ontogeny of behavioural individuality is thought to have the potential to explain the evolutionary history of division of labour (Ross and Matthews 1991). Despite this fact, few studies have focused on inter-individual variation *per se* (Hurd et al. 2003; Tenczar et al. 2014), and even fewer have monitored individuals' activity over their entire lifetime.

We found extreme intra-colonial differences in the level of foraging activity (Fig. 3.2, 3.3, 3.S5, Table 3.1) and task specialization of common wasp workers (Fig. 3.1, Table 3.1), consistent and significant over the entire life of the wasps. Within the same cohorts and colonies, some “elite” *V. vulgaris* workers started foraging on the very first day of life and performed a particularly impressive number of foraging trips throughout their life, especially when compared to RFID monitored *A. mellifera* (Fig. 3.S5) (Perry et al. 2015). Some other workers lived relatively long, but performed very few trips in their lifetime or never foraged. Wasp individuals showed all degrees of specialization in relation to alternative foraging tasks, with some wasps dedicating their whole life to the almost exclusive collection of flesh, pulp or fluids. Great differences in the activity level of nestmates have been noted in numerous insect societies, mostly via casual observations (Sommeijer et al. 1983; Jeanne 1991; Biesmeijer and Tóth 1998; Spaethe and Weidenmüller 2002; Polidori et al. 2006; Tenczar et al. 2014; Charbonneau and Dornhaus 2015). Fidelity to particular foraging tasks has been previously observed in worker individuals of wasps (West-Eberhard 1969; O'Donnell and Jeanne 1990; Archer 2004), hornets (Matsuura 1975), bumble bees (Spaethe and Weidenmüller 2002; Hagbery and Nieh 2012), stingless bees

(Sommeijer et al. 1983; Biesmeijer and Tóth 1998), honey bees (Calderone and Page 1988; Oldroyd et al. 1992) and ants (Beverly et al. 2009), although not always (e.g. Kolmes and Sommeijer 1992; Jandt et al. 2009). In the vast majority of these studies, though, any speculation as to causation was limited for a number of reasons, including small sample sizes, short monitoring intervals over individual lifespans, sampling method biases, and artificial feeding conditions. Individual specialization on critically short time scales might be the simple by-product of transient resource availability, or result from the characteristic site-fidelity and exploitation of the same individual resource (e.g. a single large prey item brought back to the nest in numerous consecutive trips) (Raveret Richter 2000).

What is the source of the tremendous variability found in the common wasps' lifetime foraging behaviour? What can explain "elite" or "specialist" individuals? In general, the causes of inter-individual variability in social insects can be ascribed to genetic diversity and phenotypic plasticity mechanisms (for a review see Jeanson and Weidenmüller 2013). A variety of factors, most likely interacting, have been linked to foraging behaviour variability. These include social dynamics, group size, colony phenology, individual reproductive ability, past foraging experience, genotype, personality, age, size, spatial location within the nest, pathogen load, and life expectancy (Potter 1964; Seeley 1982; Robinson 1992a; Hurd et al. 2003; Grinsted et al. 2013; Kuszewska and Woyciechowski 2013; Willmer and Finlayson 2014). Although generalisations are constrained by the limited sample size (three colonies), we found different degrees of inter-individual variation within workforces and the proportion of workers foraging and the foragers' effort tended to decrease with increasing colony size (Fig. 3.2, Table 3.1).

There is empirical and theoretical evidence for an effect of social status and colony size on inter-individual variation (e.g. Strassmann et al. 1984; Gautrais and Theraulaz 2002; Magnhagen and Bunnefeld 2009). Aggressive in-

interactions among nestmates could force subordinate individuals to perform riskier activities such as foraging (O'Donnell 2003), and aggression and “mauling” among *Vespula* workers is common (Spradbery 1973, Akre et al. 1976). In vertebrates, individuals in bigger groups were found to show increased behavioural difference (reviewed in Magnhagen and Bunnefeld 2009), and social insect workers in smaller colonies were observed switching tasks more often (Jeanne 1986). It was also noticed that insect workers in smaller colonies (or earlier in the season) start foraging earlier and can be more active than adults emerging later and among more nestmates (Potter 1964; Strassmann et al. 1984). These changes could be explained by higher foraging pressures earlier in the season (e.g. for food, lower worker/larval ratio – Potter 1964), or increasing advantages of selfish behaviour and personal reproduction (possible also for workers) in bigger colonies (Strassmann et al. 1984). Wasps of the genus *Polistes* are characterized by behavioural reproductive caste determination. Vespine wasp workers might similarly modulate their foraging effort in accordance to alternative strategies for maximising personal fitness (Tibbetts 2007).

As in other social wasps (Sumner et al. 2007), we observed workers drifting between colonies, confirming one earlier report of intra and inter-specific drifting within the genus *Vespula* (Akre et al. 1976). Inter-colonial differences in this study (Table 3.1) could be at least partially explained by the death of the queen in the biggest colony, C. This event, not uncommon in nature, especially towards the end of the colony cycle (Ross and Matthews 1991), was followed by an expected drop in the colony foraging activity (Potter 1964; Edwards 1980). Also, coherently with kin selection theory (Ross and Matthews 1991) and one previous experiment with *Vespula* (Hurd et al. 2007), foreign, unrelated workers introduced into colony A contributed less to foraging activities (Fig. 3.2). Further speculations on this topic are limited by the fact that the introduction of foreign workers was performed late in the season.

Individual experience and learning processes might be important in shaping alternative behaviours and increasing foraging success and level of activity.

Insects can learn from their previous experiences, particularly in the context of foraging (e.g. searching tactics, resource location, foraging routes, specific prey discovery, submission and handling techniques), and acquired skills can possibly enhance foraging performance (Free 1970; Spradbery 1973; Schmid-Hempel 1984; Punzo 2005; Ravary et al. 2007; Raine and Chittka 2008). For example, the prey-hunting success of *V. pensylvanica* and *V. atropilosa* was reported to be very low in the first foraging days (1:10) and to increase with age (Akre et al. 1976), similarly to the foraging success rate of *Polybia occidentalis* wasps (O'Donnell and Jeanne 1992). Younger *V. vulgaris* workers observed in the present study were more likely to return to the nest with no or half fluid loads, while older individuals returned more often with full fluid loads. This difference is not trivial, in terms of net food intake and foraging success, given that crop liquid weight of *V. vulgaris* full fluid carriers was found to be 3-8 times that of partial liquid carriers (Archer 1977). Older, more experienced workers foraged more throughout their lives. Our continuous monitoring revealed that the number of foraging trips performed by individuals increased with age for most of their lives, in contrast to what has been repeatedly reported so far in different species of wasps (Potter 1964; Spradbery 1973; Edwards 1980; O'Donnell and Jeanne 1992b). Contrary to what has been argued in the past for *V. germanica* (Hurd et al. 2007), *V. vulgaris* foragers have many occasions to learn, when compared, for example, to honey bees. In fact, aside from the much higher average number of lifetime trips, common wasps can show comparable lifespans and even longer foraging tenure length than honey bees during spring and summer (Sakagami and Fukuda 1968; Neukirch 1982), also due to the wasps early foraging onset (Fig. 3.S5).

The spectacular variability in foraging that we observed among wasp workers could be mirroring intrinsic differences among individuals, which awaits to be tested. Wasps foraging earlier, foraging more or spending more time outside the nest could be innately bolder and more inclined to risk-taking behaviours. Recent literature is starting to demonstrate how innate individual personality traits such as boldness and aggressiveness can vary within colonies

(Grinsted et al. 2013; Santoro et al. 2015) and play a role both in food intake rates and in social organization (Biro and Stamps 2008; Grinsted et al. 2013).

Heritable influences on the division of labour within insect societies are more important and pervasive than previously thought (Smith et al. 2008). For example, a genetic component in foraging task specialization was found in *Polybia aequatorialis* wasps (O'Donnell 1996). Gene expression accompanies the transition to foraging activities in *V. vulgaris* wasps, *Pogonomyrmex barbatus* ants and *A. mellifera* (Tobback et al. 2008). In honey bees, behavioural traits that have a demonstrated genetic component include stinging, foraging for pollen or nectar, undertaking, and learning (Oldroyd et al. 1992; Robinson 1992; Hunt et al. 1998). Polyandry is hence thought to have an adaptive significance and a role in division of labour, determining worker subgroups with different task-related thresholds (Oldroyd and Fewell 2007; Goodisman et al. 2007; Jeanson and Weidenmüller 2013).

For individual workers, changes in task preference are also caused by epigenetic changes over their life. Honey bees of different ages and ants of different worker castes perform different tasks, and show substantial differences in DNA methylation, causing differences in gene expression (Herb et al. 2012, Simola et al. 2016). Some wasp workers could inherit from the queen or alternative patrines genes responsible for highly bold, exploratory behaviours, expressing during their adult life. Individuals with innately bolder personalities would be more likely to undertake risk-taking activities such as foraging earlier, and maintain high levels of activity over their entire life (Wolf 2007; Biro and Stamp 2008). Since for eusocial insect workers fitness is almost exclusively inclusive, even suicidal behaviours are adaptive (Shorter and Rueppell, 2012). Extreme behavioural phenotypes, and increased inter-individual variation might be hence promoted and more frequent within highly social animal groups.

3.5.2 Polyethism by age

The only previous study examining age polyethism in *V. vulgaris* (Potter 1964) has been used for decades to exemplify temporal changes in behaviour in the genus, and social wasps in general (Spradbery 1973; Edwards 1980), but was limited by a small sample size (Fig. 1.4). We re-examined the age-related variation in foraging activity levels and foraging activity changes in the common wasp. The temporal polyethism observed in our wasp colonies strengthens the previous findings for the species and highlights inter-specific similarities within the genus *Vespula* (Potter 1964; Akre et al. 1976; Hurd et al. 2007; Kim et al. 2012). Fluid collection increased with age and, between solid loads, pulp loads prevailed earlier, flesh loads later in life. These type of temporal transitions in workers' foraging behaviour were not found in other Vespinae with smaller colonies such as *Vespa* (Matsuura 1975) and appear to be clearer in the swarm-founding Polistinae with bigger colonies such as *Agelaia* (Jeanne 1991). We have above discussed these temporal patterns both under the perspective of group size and individual experience. The differences in the workers' age when performing for the first time the alternative foraging tasks were restricted to 3 to 5 days (colonies C and B) time windows (Fig. 3.4), and could be considered of minor biological significance (Hurd et al. 2007), especially when considering the above described variability among individuals. Yet, their importance could be underestimated when considered in relation to the foraging tenure length, which we observed to be between 3.5 and 16.5 days (colonies C and B) (Table 3.1). There is a suggestive similarity between workers' and foundress queens' temporal sequence of foraging activities, perhaps supporting the hypothesis of a genetically set biological clock (Spradbery 1973; Ross and Matthews 1991; Tobback et al. 2008). An additional, poorly explored hypothesis is that temporal changes in individual foraging behaviour mirror changes in the colony needs.

3.5.3 Polyethism by size

We investigated the role of individual size on foraging behaviour, hypothesizing that relatively larger workers within a colony are better foragers and that size influences the foraging task preferentially performed. We found that bigger wasp workers are more likely to forage, start foraging earlier and tend to be better foragers, tending to accomplish foraging trips in a shorter time or perform more trips per foraging day. Moreover, individuals returning to the nest relatively more often with flesh loads tended to be larger. These findings reinforce evidence of size-related foraging advantages reported in *Vespula* previously (Spradbery 1973; Hurd et al. 2007). Size is a key factor influencing foraging performance in non-obvious ways (MacNulty et al., 2009; Santoro et al., 2011). Bigger individuals have been repeatedly found to be better foragers, particularly among predators, facing fewer limitations in relation to the relative prey size (Cogni, 2002; MacNulty et al., 2009; Polidori et al., 2011; Santoro et al., 2011). Bumble bees can vary greatly in size; foraging individuals were found to be bigger than the average workers' size within a nest (Hagbery and Nieh 2012). Larger workers also showed increased olfactory sensitivity and hence resource detection ability (Spaethe et al. 2007). Size-related differences in foraging behaviour were also reported for the morphologically less variable honey bees, with bigger workers tending to forage more frequently (Cideciyan 1984, in Waddington 1989).

Aside from the foraging activity measures considered here, larger workers can contribute more to the colony foraging economies in alternative ways, for example carrying heavier loads on each trip. Bigger individuals can carry heavier loads in other wasp species (Santoro et al. 2011). Increased carrying capability associated with individual body size could be particularly important for fluids, since fluid collection for social wasps normally takes longer (Matsuura 1975; Archer 2012; Kim et al. 2012), fluid loads are the heaviest (Archer 1977; O'Donnell 1995; Kim et al. 2012) and are ultimately limited by crop capacity (Jeanne 1986). The greater carrying capacity of large workers could, for example,

explain the disproportionate contribution to the nectar economy of bigger *Bombus* foragers, as trip number, trip length and proportion of nectar trips were the same as smaller nestmates (Spaethe and Weidenmüller 2002). Contrary to solitary wasps (Santoro et al. 2011), social wasps are not constrained in the transport of prey for the ability to dismember and malaxate the prey (Polidori et al. 2013). Larger social wasps could still have an advantage in hunting activities, possibly being able to attack and subdue bigger prey. In solitary wasps, bigger individuals normally win in territorial disputes among conspecific males (Asís et al. 2005). In inter-specific competitive interactions, smaller social wasps avoid resources occupied by bigger wasps (Matsuura & Yamane, 1990; Raveret Richter & Tisch, 1999). Hence, larger workers of social wasps could also have preferential access to shared resources, for which inter-individual direct competition is the norm (Parrish 1984; Raveret Richter 1990).

3.5.4 Survival

Using automated RFID monitoring data, we studied the lifespan and survival of large numbers of workers and foragers of the common wasp *V. vulgaris*. The wasps' lifespan was highly variable, within and between colonies, and the maximum lifespan recorded (50 days) exceeded by 2 weeks the longest lifespan previously reported in these wasps (Archer 1981, in Strassmann 1985). The life expectancy of the workers from the colony that lost their queen (colony C) was half that of the workers in the healthy colony (B). The shorter lifespan could be explained by the observed rise of cannibalism, as reported in previous literature (Spradbery 1973), although alternative factors such as different rearing and laboratory conditions might have had a role. It might also represent a substantial underestimate, biased by some of the workers leaving the nest to find other colonies. A tight relationship between the destiny of the colony and the mortality of workers has been observed in other social wasps of the genus *Polistes* (Strassmann 1985). The highest worker mortality rates corresponded to the foraging onset time window (Fig. 3.7), and a general trade-off between foraging

onset age and longevity emerged, as also suggested by the shorter lifespan of the big workers from colony B, starting to forage significantly earlier than their nest-mates.

Our results have added to the literature demonstrating that foraging appears to be a very risky activity, for common wasps as for other species of wasps (Strassmann et al. 1984; Strassmann 1985; O'Donnell and Jeanne 1992a; Torres et al. 2013) and hymenopterans in general (Neukirch 1982; Biesmeijer and Tóth 1998). In honey bees, foragers exhaust their energies after a definite flight performance, showing accelerated consumption of lipids, senescence and premature death compared to non-foraging individuals (Neukirch 1982; Remolina et al. 2007). Honey bee workers born at the beginning of the foraging season have much shorter lives than wintering workers (on average, 23.4 vs 154 days respectively (Sakagami and Fukuda 1968), 14 to 42 vs 140 days (Remolina et al. 2007)). Earlier onset of foraging has been recently found to be associated with premature death and linked to colony collapse in honey bees (Perry et al. 2015). Despite being longer than previously reported, *V. vulgaris* average lifespan is still shorter than that of more primitively social wasps such as *Polistes* (Strassmann 1985; Torres et al. 2013).

As RFID technology becomes more widely available we look forward to an abundance of individual-level, lifetime data from a range of social insect species, in order to increase our understanding of how the complex of individual behaviours translates into colony success and failure.

3.6 Supplementary material

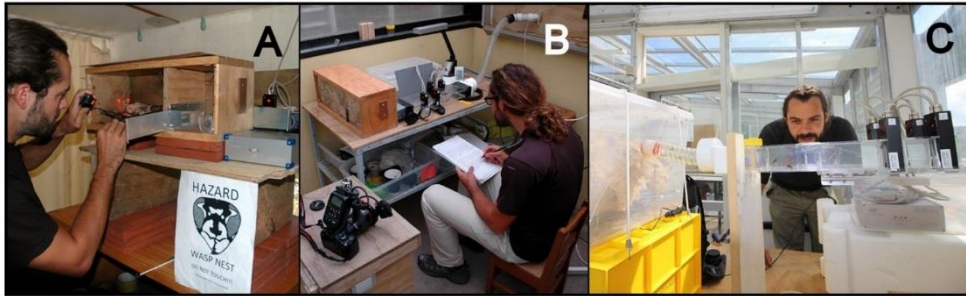


Fig. 3.S1 *Vespula vulgaris* colonies set up in 2013 (A), 2014 (B) and 2015 (C). After being collected in the field, the nests were kept in boxes in three different study sites (2013, 2015: Wellington; 2014: Lincoln, New Zealand). Individually tagged wasps were free to forage in the field passing by a transparent nest entrance provided with a radio-frequency identification (RFID) system.

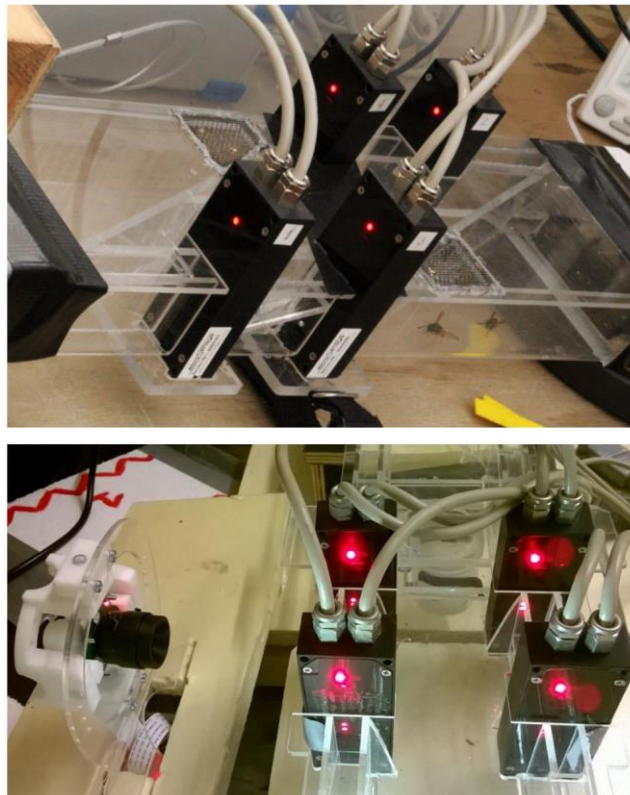


Fig. 3.S2 The double-funnel nest entrance, directing wasps' outgoers and incomers in two separated lanes. The entrance was provided with RFID tunnel readers to automatically record exact time and identity of the workers with RFID tags walking by. Incoming wasps were continuously filmed with a raspberry picam.

3. The Common Wasp Radio-Tagged

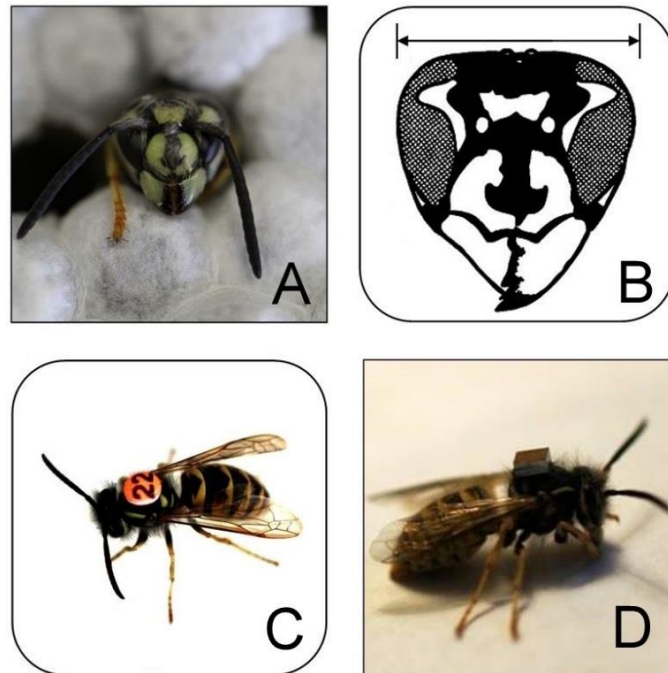


Fig. 3.S3 *Vespula vulgaris* (A) adult worker emerging from an incubated comb, after 17-36 days since the mother queen laid the egg and passing by five larval instars and 8-18 days of pupation (Spradbery 1973) (B) head width measure as a proxy of individual size (Polidori et al. 2011) (C) individually marked with numbered plastic tag (2mg approx weight) (D) provided with RFID tag (microsensys, 5mg approx weight).



Fig. 3.S4 *Vespula vulgaris* workers returning to their colony from foraging trips. The difference in size between wasp nestmates can be striking. On the left of the picture and in the in-built box, an individual with a radio-frequency identification (RFID) tag glued on the thorax and painted on the abdomen is visible.

3. The Common Wasp Radio-Tagged

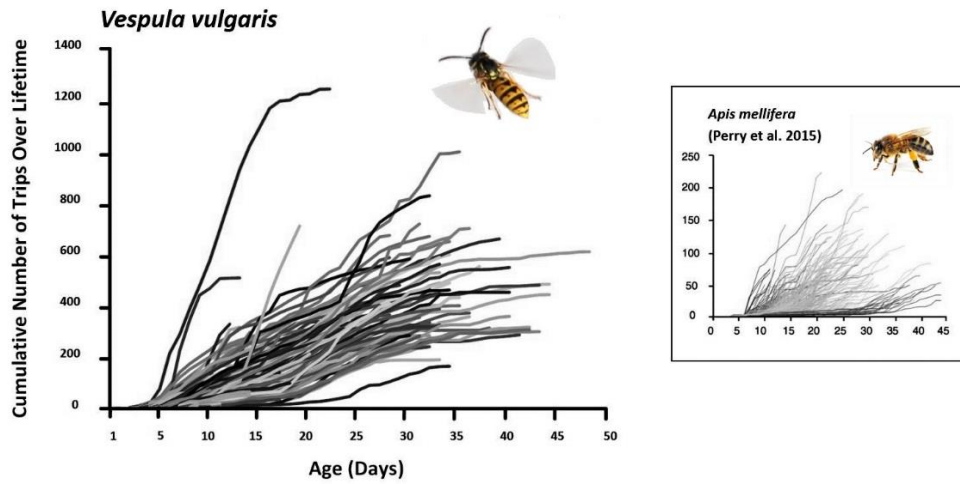


Fig. 3.S5 Cumulative number of lifetime trips in the common wasp *Vespula vulgaris* (data from colony B, this study) and the honey bee *Apis mellifera* (after Perry et al. 2015). RFID data were filtered following the same criteria. The extreme inter-individual variation in foraging activity is evident in both species. Comparatively, wasp foragers can perform many more foraging trips and can show similar lifespans. Foraging onset age is slightly anticipated and appears less variable in wasps.

Chapter 4

Behaviourally Specialized Workers Perform Better and Live Longer in an Insect Society

4.1 Abstract

We directly test the fundamental assumption in biological studies that specialists are more efficient than generalists. The consequences of specialization were investigated in the foraging context, by comparing closely related individuals experiencing the same physical and social environment, nestmates from the same insect colony. We applied indices of niche overlap to measure the degree of division of labour and relative individual task specialization in a social insect, the common wasp *Vespula vulgaris*. Using radio-frequency identification technology, we monitored the foraging activity of individual wasps, to measure their overall lifetime performance and survival in natural foraging conditions. Returning foragers were recorded throughout their adult life when passing the nest entrance and the task allocation at the individual and colony level could be assessed. We found a significant level of foraging task specialization, and specialist foragers were more efficient than generalists within the same colony. There were large differences in activity and foraging efficiency among individuals, which were not explained by variation in age or size. Behavioural specialists performed more trips per foraging day and their trips were relatively shorter. Specialized foragers lived and foraged for a significantly longer time. This study provides empirical evidence that the behavioural specialization of monomorphic individuals is associated to a better life-time performance and suggests that division of labour in a social insect colony can have adaptive benefits via increased worker foraging efficiency.

Keywords *Vespula vulgaris*, foraging, efficiency, division of labour, niche overlap

4.2 Introduction

One fundamental assumption in ecological and evolutionary studies is that specialists are more efficient than generalists (Sloan Wilson and Yoshimura 1994). This critical assumption implies that specialists evolved physiological, morphological or behavioural traits leading to greater efficiency in specific resource exploitation (Drummond 1983; Futuyma and Moreno 1988; Ferry-Graham et al. 2002). The investigation of fitness consequences of ecological specialization is still very limited, especially amongst genetically highly related individuals (Bolnick et al. 2003; Woo et al. 2008). In the context of foraging, the feeding specialization hypothesis (alias diet specificity (Whitfield et al. 2009)), suggesting that specialist should show higher foraging efficiency than generalists, has a long history, dating back at least to Dethier (1954, in Price 1997). The hypothesis has been historically tested at the species level, though often with contradictory results (Strickler 1979; Drummond 1983; Bolnick et al. 2003). During the 1980's, after considerable accumulation of comparative studies among species, it was noted that variation in rates and efficiencies of food utilization is mostly independent of the degree of specialization, at least among insects (Slansky and Scriber 1985, in Price 1997).

Specialization can be defined according to different conceptual frameworks and investigated at different levels (Ferry-Graham et al. 2002). The evolution of specialized behaviour must be distinguished from the evolution of morphological and physiological traits (Futuyma and Moreno 1988). Surprisingly, few studies have tested and empirically demonstrated that different species/populations with distinct behavioural phenotypes show different efficiencies. Some empirical evidences that specialists have evolved behavioural adaptations to handle their preferred food types more efficiently were found (Strickler 1979; Drummond 1983; Lavery and Plowright 1988). The significance of behavioural specialization was alternatively investigated within species, at the individual level, and its link with increased performance has found some empirical support in

invertebrates and vertebrates (Bernays and Funk 1999; Rana et al. 2002; Whitfield et al. 2009).

A growing body of literature is providing evidence that individual animals, similarly to humans, vary greatly in their behaviour and personality (Wolf et al. 2007). Animal personality traits (PTs) can be defined as “behavioural tendencies that affect behaviour in several different contexts, vary across the individuals in a given population and are consistent within individuals across time” (in Biro & Stamp 2008). Exemplary traits are activity, shyness, boldness, exploration, aggressiveness and sociability (in Biro & Stamp 2008). Yet, the animal personality debate is controversial, for personality traits can either be considered as the by-products of ecological constraints or as adaptive responses under direct selection (Wolf et al. 2007). Life history theory predicts that individuals should adjust their risk-taking behaviour to their residual reproductive value (Dingemanse and Wolf 2013). The most relevant hypotheses to explain the evolution, development and maintenance of PTs assume that inter-individual variation in PTs is maintained as a result of life history trade-offs that involve PTs (Biro & Stamp 2008). As a consequence, older individuals are expected to take riskier decisions or, in a species in which both activity and boldness are positively related to food intake rates, individuals with consistently higher growth rates or fecundity should display consistently high levels of activity and boldness (Biro & Stamp 2008).

The study of PTs in social insects can give unique insights on their ecological or adaptive significance, since in these societies selection operates both at the individual and the colony level. Animal societies, mostly represented by social insects, are based on division of labour. Task differentiation is a necessary evolutionary step and is considered to be a major cause for the ecological success of eusocial insects (Wilson 1975; Chittka and Müller 2009; Jeanson and Weidenmüller 2013). Theoretically, to develop, evolve and to be maintained, the differential task allocation among individuals and their consequent specialization must increase colony fitness. Increased efficiency at the colony level can be

achieved both via group task partitioning advantages and via increased individual efficiency in task performance, two mutually non-exclusive mechanisms (Jeanne 1986; Goldsby et al. 2012).

There is increasing evidence that there are enormous differences in activity among insect nestmates (O'Donnell and Jeanne 1992a; Hurd et al. 2003; Polidori et al. 2006; Perry et al. 2015). Some individuals, referred to as “elite workers” are extremely active and productive in tasks foraging (Hurd et al. 2003; Perry et al. 2015), while others are relatively “lazy” (Charbonneau and Dornhaus 2015). We are still far from understanding the causes and consequences of these huge inter-individual differences (Jeanne 1988; Ross and Matthews 1991; Dornhaus 2008; Chittka and Muller 2009). For the colony, foraging is a fundamental requirement to grow in size and strength (producing workers) and reproduce (producing males and gynes). For the individual worker, foraging is a risky and costly activity (O'Donnell and Jeanne 1992b; Biesmeijer and Tóth 1998; Wolf and Schmid-Hempel 1989; Robinson et al. 2009;) which might result in accelerated senescence and premature death (Neukirch 1982; Wolf and Schmid-Hempel 1989; Remolina et al. 2007). Hence, individual foraging enhances colony fitness at the expense of a forager's relative intra-colony fitness (Jeanne 1991). Social insect workers specializing in a particular task are commonly assumed to be more efficient than those individuals often switching task in response to the changing colony needs (Dornhaus 2008; Jeanson and Weidenmüller 2013; Jeanson and Lachaud 2015). Very few studies, however, have investigated the efficiency of individuals and how it relates to which task they perform (Dornhaus 2008; Chittka and Muller 2009; Duarte et al. 2011).

In the present paper, we quantify the degree of division of labour and task specialization in a social insect, using the common wasp *Vespula vulgaris* as a model organism. Combining longitudinal observational data and lifetime data obtained through automated monitoring, we focus on the consequences of foraging task specialization on the individual-level performance, to investigate

whether individual specialization can predict efficiency. We hence addressed the following questions: (i) Do *V. vulgaris* workers specialize on a particular foraging task, throughout their life, and to what degree? (ii) Are specialized foragers more efficient than generalist nestmates? (iii) Is foraging specialism linked to individual survival?

4.3 Materials and methods

4.3.1 Study organism

The common wasp *Vespula vulgaris* (Hymenoptera: Vespidae) is an eusocial insect species native to Eurasia and has become a notorious pest in countries such as Argentina and New Zealand, attaining high densities and causing major ecological impacts in the invaded range (Lester et al. 2014). Common wasps forage for pulp, water, carbohydrates and proteins. Pulp is used as a nest construction material, both for the combs and the envelope. Water is used in combination with pulp for nest construction and for colony thermal regulation. Carbohydrates are obtained from sources such as nectar or honeydew. Proteins come from hunting and scavenging activities (the latter being an almost unique feature of the *V. vulgaris* species group, among wasps). Flesh pieces are fed to the larvae by adults (Archer 2012). *Vespula* workers are monomorphic but can show important differences in size (Spradbery 1972). There is some evidence that *Vespula* workers can specialize in a particular foraging task on different time scales (Brian and Brian 1952; Hurd et al. 2007).

4.3.2 Experimental set up

The study was conducted during 2014, in New Zealand. One colony of *V. vulgaris* was collected on 20 January from Christchurch, New Zealand. About 450 workers and the queen were captured. The nest was composed of six combs (maximum diameter 17 cm), for a total of approximately 1500 cells (combs' volume 2000 cm³). On the same day, it was carried to the designed research facility

(greenhouse, Plant & Food Research Ltd, Lincoln). The colony was anesthetized with carbon dioxide and relocated into a wooden box (11000 cm³) with an exit/entrance to the outside environment.

The wasps were allowed to freely forage in the field for the following nine weeks, in “natural conditions”, passing through a transparent perspex entrance. The entrance module was designed on the basis of Potter’s (1964) drawings, 40 cm long and shaped as a double funnel to direct the traffic into two separate lanes, one for the wasps going out (“outgoers”) and one for those returning from foraging trips (“incomers”). The module fitted one RFID system (microsensys GmbH, iID®HOSTtypeMAYA4.1). Two RFID tunnel scanners (iID®MAYAreadermodule4) were placed on each lane, to record precise information on the direction taken by the individuals walking in and out of the entrance (Fig. 4.S1).

In total, 630 adult wasps were individually marked and measured. During the nest manipulation, two combs with capped brood were kept aside in an incubator (30°C, 50% relative humidity, and complete darkness) as a source of known-age workers. Between 21/1/2014 and 31/1/2014, 300 adults emerging from the incubated combs were provided with RFID tags, painted on the first abdominal tergite, and introduced in the colony of origin within 24 hours from emergence. Additional 130 known-age workers (between 1/2 and 6/2) and 200 foragers (between 29/1 and 11/2) were individually marked with plastic numbered tags (queen numbering kit, Ecrotek Ltd) and introduced in the colony. To estimate the body size of the wasps, we measured their head width, using a digital calliper to the nearest 0.01 mm.

The nest was filmed and observed every day from 28/1/2014 to 28/2/2014, and on alternate days until 17/3/2014, for a total of 145 hours of colony activity. For one-hour focal periods (8 AM to 8 PM, 61 hours from 10 AM to noon), loads carried by individually marked wasps were observed and recorded, followed by a 10 minute interval during which loads of all individuals (marked or unmarked) were recorded. During the focal observations, whenever

a wasp with RFID tag was passing by the first of the two readers in the incomers' lane (and the individual's identity and exact time automatically recorded), the resource item and time to the nearest second were noted (e.g. Fig. 4.1, 4.S1). Observational load records were subsequently matched manually with RFID traffic data from the Maja reader, and each resource item was attributed to an individual on the basis of the timestamp and colour on the wasp's abdomen.

The nest box had a glass wall that allowed monitoring of the colony, which grew in size during the season. After the death of the last tagged workers, the nest was opened and examined. The envelope completely filled the nest box, the combs were expanded (7000 cm³), and queen cells (about 300, uncapped) were built, for a total of about 5400 cells.



Fig. 4.1 *Vespa vulgaris* worker provided with RFID tag (glued on the thoracic scutum on the day of its emergence as an adult), and painted on abdominal segment. The wasp is filmed while returning from a foraging trip, walking through the nest entrance and carrying a flesh load (insect abdomen) that will be fed to the larvae.

4.3.3 Task specialization measures

We used ecological specialization indices to quantify the degree of division of labour at the colony level and lifetime task specialization of individual workers, by taking into account the task allocation at the level of their colony.

We used the Shannon index approximation of the proportion of within-individual component of total niche width (WIC/TNW), the mean proportional similarity between individuals and colony (IS), and the mean likelihood of individual's task partitioning being drawn from the task partitioning at the colony level (W_i) (Bolnick et al. 2002). These indices, traditionally used in species level niche studies, were adapted at the individual level to quantify diet specialization. The indices are highly correlated and measure the degree of niche overlap between each individual and the population (colony) (Bolnick et al. 2002). Indices' values range from 0 to 1. In general, the lower the value, the less overlap among individuals and hence the higher the degree of specialization (see Statistics section and Supplementary material, Fig. 4.S2, 4.S3 for further details).

Using Indspec1.exe (Bolnick et al. 2002), we calculated the three indices treating each type of load brought to the nest by foraging wasp workers as a food item category (fluid, pulp, flesh) and considered the colony as one population. WIC/TNW and IS were used to measure the degree of division of labour and test whether there was significant structure to foraging labour. To quantify individual specialization of each foraging wasp and distinguish “specialists” from “generalists”, we used the Petraitis' (1979) niche overlap index W_i (see Statistics section). By measuring the degree of overlap between the task allocation of each individual and the task allocation at the colony level, we obtained a measure of contextual, colony specific individual foraging task specialization. For example, consider one wasp consistently foraging on pulp: this foraging behaviour could be considered by itself “specialized”. If we took in consideration the division of labour at the colony level and we knew that the vast majority of workers were performing the same foraging activity, the individual would likely be a “generalist”. Conversely, the same individual would be a “specialist” if the same behaviour was shown in a colony with a weak pulp economy (Please see Fig. 4S.2 for additional examples).

4.3.4 Individual efficiency measures

For the known-age workers provided with RFID tags, it was possible to determine an estimate of adult lifespan (from the day of emergence to the day of last RFID record), foraging onset (age at first foraging trip) foraging tenure (number of foraging days), and number of lifetime trips. It was also possible to calculate the length of each foraging trip. RFID data filtering and handling was performed with Track-a-Bee1.0.exe (cut-off settings: cluster = 20 sec, in/out = 20 sec, flight minimal = 10 sec, flight maximal = unlimited time) (Van Geystelen, personal communication). We measured individual efficiency and foraging performance considering the *average number of trips per foraging day (ANTFD)*. This measure was preferred to the number of lifetime foraging trips because, for example, some individuals might do more trips and look more “efficient” simply because they lived longer and/or had a longer foraging tenure than others (see Results section).

We also measured efficiency as a function of trip length, elaborating a standardized measure. Given that the duration per foraging trip reflects how much is accomplished per time, more performing individuals would do shorter trips (e. g. Dornhaus 2008). Trip length could vary in time and different resources could require different collecting times (Brian and Brian 1952; Potter 1964). As expected, especially in natural foraging conditions, we found enormous variability in the length of the foraging trips performed by the same individual throughout its life and among different individuals at any one time.

Because of factors such as weather conditions, a standardized measure of trip length was needed. First, to control for trip length variation in time, we calculated for each individual i the *average relative trip length (ARTL_i)*, measured as the average of all the trip lengths, each one divided by the average trip length of all the active foragers at any corresponding day and hour. Second, to take into account the different time needed to obtain different load types, we calculated the *average relative trip length (ARTL_j)* for each load type j (fluid, pulp, flesh), dividing the average trip length (ATL) for each load type (ATL_j) by the lowest

(ATL_{j1}) ($ARTL_{j1} = 1$; $ARTL_j > 1$). Then, to take into account the individual task allocation and its potential influence on the ATL, an individual correction factor K_i was calculated as $K_i = \sum P_{ij} * ATL_j$, where P_{ij} is the proportion of load type j for the individual i . Finally, the individual *average standard trip length* ($ASTL_i$) was calculated as $ASTL_i = K_i * ARTL_i$. Hence, $ASTL_i$ represented a measure of the average lifetime performance of each individual across any one time period in comparison to the simultaneous performance of all the other foragers, controlling for the differential individual task allocation.

4.3.5 Statistics

To test whether the degree of specialization measured by WIC/TNW and IS represented a significant level of division of labour in foraging activities among workers, we used Indspec1.exe (Bolnick et al. 2002). The program uses a nonparametric Monte Carlo procedure to generate replicate null matrices from the population distribution (where matrix rows are individuals, and columns foraging tasks - row and column totals are constrained), from which p values can be computed. Ten thousand replicates in Monte Carlo bootstrap simulations were performed. W_i allows a parametric statistical test of the null hypothesis that an individual draws randomly from a population (Bolnick et al. 2002). Indspec1.exe generates and associates probability measures to each individual score. On the basis of the probability values obtained, we separated the foragers in two foraging status groups, worker “specialists” ($W_i p < 0.05$) and “generalists” ($W_i p > 0.05$). To test whether the two groups of workers showed different foraging efficiency, measured as ANTFD or ASTL, we performed non-parametric Mann-Whitney test. The same was done to test for differences in average size and foraging onset between specialists and generalists. For this purpose, we used R 3.1.0 (R Development Core Team 2013). A Kaplan-Meier survival curve and log rank statistic were used to compare mortality between specialists and generalists. The survival analysis was performed with IBM SPSS Statistics 21 (IBM Corp. 2012).

4.4 Results

4.4.1 Individual foraging task specialization (observational data)

Overall, the outcome of 25925 foraging trips was observed. The prevalent foraging task at the colony level was represented by fluid foraging, with 63.2% of the returning foragers showing a swollen abdomen, having either a partially full ($n = 5437$) or full crop ($n = 10618$) and no solid loads in the mandibles (Fig. 4.2, 4.S2). Pulp loads accounted for the 20.4% of all the loads (Fig. 4.2, 4.S2). Flesh was carried by 16.3% of the incoming foragers (Fig. 4.1, 4.2, 4.S2). In 2.1% of the cases, incomers were observed with no solid loads and empty crops. A subset of 15521 loads was attributable to 456 individually marked wasps, and 8257 of these loads were carried by 186 individuals provided with RFID tag (see below).

There were substantial differences between individual foraging careers. Most of the individuals were seen performing the three foraging tasks, but wasp workers showed foraging task distributions covering almost the whole spectrum theoretically possible, with some individuals seen performing only one foraging task throughout their adult life (Fig. 4.2). Individuals also varied enormously in their overall contribution to the colony foraging economy. The number of individual trips observed ranged from 1 to 201 (Fig. 4.2) mirrored actual differences recorded with the RFID system (see following section, Fig. 4.3a).

Colony labour was significantly structured, being the average individual task allocation very unlikely to be drawn randomly ($WIC/TNW = 0.7586$, $p < 0.001$; $IS = 0.7385$, $p < 0.001$).

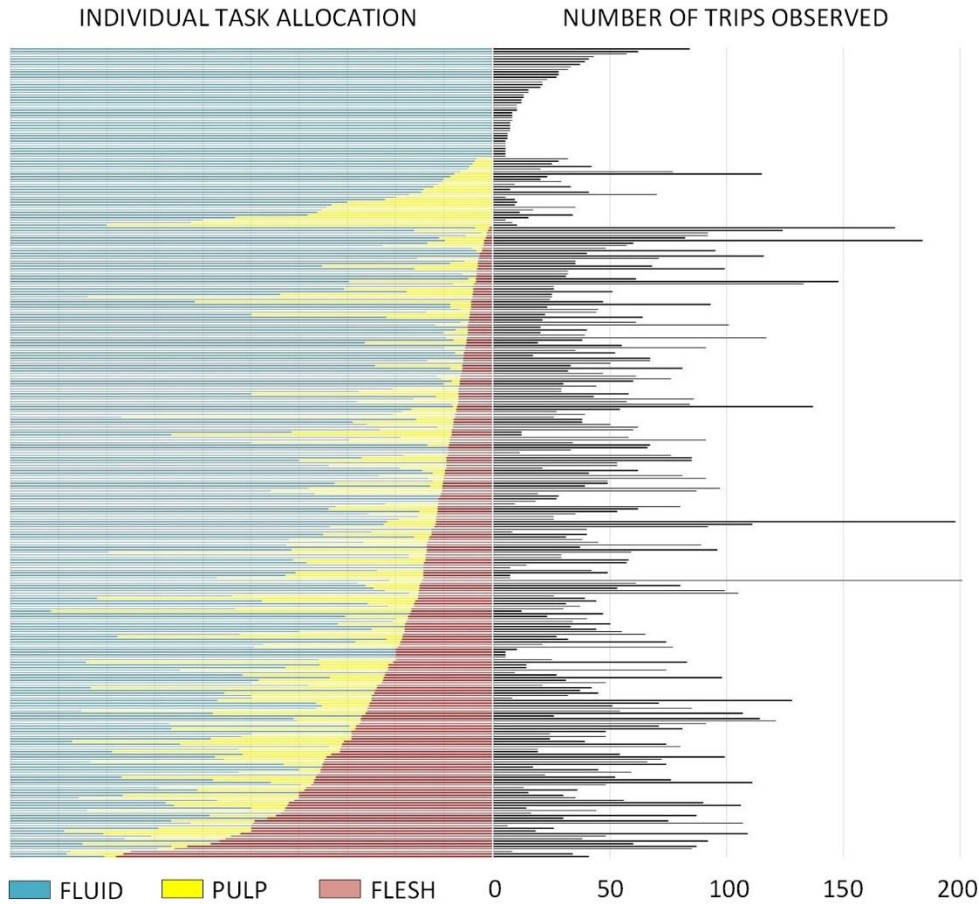


Fig. 4.2 (left) Inter-individual variation in foraging task allocation among colony members, where each horizontal line represents one individually marked common wasp worker (for the three foraging tasks here distinguished, see Plate I, page 152). (right) Number of loads observed for each individual throughout its life and foraging career (only individuals observed with more than five loads are represented).

4.4.2 Task specialization, efficiency, and survival (RFID data)

Of the 300 wasps provided with RFID tags and inserted in the nest, 14 were never recorded (They likely died in the nest before leaving it). The lifespan of the remaining adults varied from 1 to 48 days (average lifespan \pm standard deviation 18.3 ± 12.3 days). Wasps ventured out of the nest and were automatically recorded for the first time from the 1st to the 18th day of life (average foraging onset \pm standard deviation 6.0 ± 2.3 days).

We found that the time spent outside of the nest varied enormously, ranging from 20 seconds (individuals walking in a circle in the entrance) to 19 days. Wasps spending multiple days out were probably drifting between colonies, as observed for other common wasp workers by the author and from other studies on social wasps (Akre et al. 1976; Sumner et al. 2007). Half of the workers spent more than 8 hours out in the field at least once during their life, most likely overnight (the time windows roughly corresponded to the night-time foraging stop) (Spradbery 1973). To avoid confusing effects from circling, drifting and overnighing episodes, we filtered the Track-a-Bee1.0.exe output considering “foraging trips” periods of time outside of the nest longer than two minutes and shorter than eight hours (personal observations showed that 2 minutes was the minimum time necessary to walk through the entrance both ways and take a resource in front of the nest entrance). We could hence identify and measure the length of 32241 foraging trips. The load carried by the foragers was observed for 5608 trips, but in 75 cases incomers had no load.

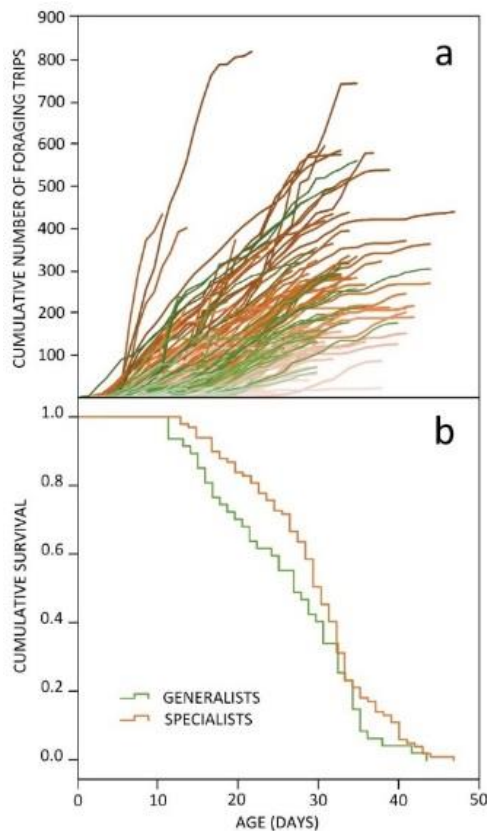


Fig. 4.3 (a) Cumulative trip number for common wasp foragers. Orange lines represent specialized individuals, green lines generalists. Colours are darker for individuals with higher number of lifetime trips. (b) Survival curves for specialists and generalists.

The trip length varied depending on the type of load (Kruskal-Wallis, $\chi^2_2 = 94.58, p < 0.001$). The average time needed to collect fluid ($n = 3446$) was 27.2 ± 23.6 min, pulp ($n = 1146$) 22.1 ± 18.2 min, and flesh ($n = 941$) 20.8 ± 19 min. Fluid collection took longer than pulp collection (Kruskal-Wallis, $\chi^2_1 = 37.92, p < 0.001$) and flesh collection (Kruskal-Wallis, $\chi^2_1 = 73.15, p < 0.001$). Trips for flesh were shorter than trips for pulp (Kruskal-Wallis, $\chi^2_1 = 14.91, p < 0.001$).

Among the wasps provided with RFID tags, about one individual out of three made no foraging trips. Between the 208 RFID tagged foragers (lifespan: 28.3 ± 8.8 , 9 – 48 days), great differences in foraging effort were found. The foraging tenure lasted from 1 to 45 days (14.2 ± 12.2). Wasps performed their first foraging trip from 1 to 23 days old. The number of lifetime trips varied enormously (average, standard deviation: 125 ± 135 ; minimum-maximum: 1–819) (Fig. 4.3a). Specialists lived, on average, 20% longer than generalists (lifespan: 30.0 ± 8.0 days, vs 25.1 ± 9.5 days; $\chi^2_1 = 5.73, p = 0.017$) (Fig. 4.3b), and the same trend was observed in another wasp colony in 2015 (Fig. 4.S4). Specialist wasps also showed longer foraging tenure ($\chi^2_1 = 8.08, p = 0.005$). On the other hand, compared to generalists (head width: 3.50 ± 0.06), specialists showed no differences in size (head width: 3.50 ± 0.06 vs 3.51 ± 0.05 ; $\chi^2_1 = 0.21, p = 0.65$) and foraging onset age ($\chi^2_1 = 0.42, p = 0.516$).

A subset of 138 foragers were observed with at least 9 loads (smaller sample sizes could potentially affect the foraging status, please see Fig. 4.S2, case 2, Fig. 4.S3) and were hence considered for the main analysis of foraging performance. Despite the huge variability observed between individuals and foraging trips, we found that specialists were more efficient than generalists according to both the efficiency measures calculated (Fig. 4.4). Specialists made significantly more trips per foraging day (higher ANTFD) averaging 63% more trips per each foraging day ($\chi^2_1 = 9.84, p = 0.002$, Fig. 4.4a). Specialists also spent 15% less time in the field for each trip (lower ASTL) ($\chi^2_1 = 6.74, p = 0.001$, Fig. 4.4b).

These results were confirmed including individuals from the same colony observed with at least 2 loads (Fig. 4.S5). Specialist wasp foragers observed with at least 2 loads in the 2015 colony also made significantly more trips per foraging day (higher ANTFD), when compared to generalist nestmates (Fig. 4.S5).

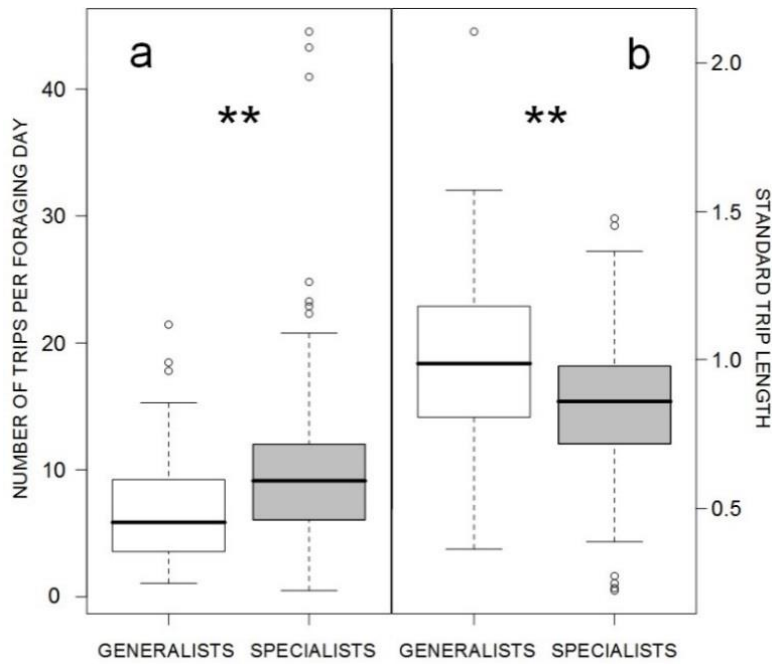


Fig. 4.4 Individual foraging specialization and performance measured as (a) average number of trips per foraging day and (b) average standard trip length. Boxes represent the lower and upper quartile, the bold line is the median and whiskers represent extreme values, with the circles identifying outliers. (a) $** \chi^2_1 = 9.84, p = 0.002$; (b) $** \chi^2_1 = 6.74, p = 0.001$.

4.5 Discussion

Behavioural specialization is widespread in generalist populations (Bolnick et al. 2003, Wolf et al. 2007) and can be considered the rule in insect societies, when referring to the propensity of individuals to perform alternative tasks (Jeanne 1988; Jeanson and Weidenmüller 2013). In the present study, we first quantified the degree of division of labour and how common task specialization is in a colony of social insects. Focusing on resource provisioning activities, we found that common wasp foraging was significantly structured among individu-

als, and that individual workers can differ enormously in their foraging behaviour and contribution to the different foraging requirements of the colony. Second, we tested whether specialized common wasp foragers are more efficient than generalist ones. Our data provided empirical evidence that the degree of foraging task specialization can indeed predict the lifetime nest provisioning performance of individual wasp workers. Finally, we found that specialists have longer lives.

Although in the majority of studies on division of labour “foraging” is regarded as a single task (Gordon 1996), it must be highlighted that the different foraging activities (i) represent the contribution to different functional task at the colony level (e.g. nest building vs larval food provisioning), (ii) respond to different stimuli in the colony environment (e.g., in *Vespula* wasps, relatively to the above examples, lighting/thermal conditions vs larval “hunger” call) (iii) can be linked to differential costs for the foragers (O’Donnell and Jeanne 1992a; Biesmeijer and Tóth 1998) and (iv) can be influenced by previous experience and learning processes (O’Donnell and Jeanne 1992b; Castellanos et al. 2004; Ravary et al. 2007; Raine and Chittka 2008; Chittka and Muller 2009). Moreover, tasks can be partitioned and task partitioning differs between and within species and colonies (Gordon, 1996; Matsuura & Yamane, 1990). Division of labour can be restructured (e.g. according to colony size, see Jeanne 1986, Jeanson and Fewell 2007) and is constantly tuned to transient environmental conditions, through feedback mechanisms mediated by signals and cues within the colony (Johnson 2010; Santoro et al. 2015a).

To show that specialization exists, it is not sufficient to quantify how much an individual performs a single task. Instead, it is necessary to show that workers preferentially perform one task compared to other nestmates and consider different tasks. It is also fundamental to focus on the different time scales across which specialization can occur (Dornhaus 2008). Compared to their nestmates, many *V. vulgaris* workers showed a preferential foraging task throughout their lives, but most of them were seen provisioning for different resources, as

previously noticed on shorter time scales with the same and in closely related species (Archer 2004; Hurd et al. 2007). In general, lifelong specialization on a single task is rare among social insects, even among ants (Gordon 1996; Dornhaus 2008).

Inter-individual variability among monomorphic insect workers in a social colony has long been known (West-Eberhard 1969; Jeanne 1988). Individual foraging task specialization (“fixation”) was reported on different time-scales for bumble bees (Hagbery and Nieh 2012), stingless bees (Inoue et al. 1985; Biesmeijer and Tóth 1998) and honey bees foragers (Oldroyd et al. 1992), with individuals consistently provisioning for pollen vs nectar or resin. Variation in foraging among individual ants is well known (Schmid-Hempel 1984; Dornhaus 2008). Among wasps and hornets, foraging differences among individuals have also been documented (Matsuura & Yamane, 1990; O’Donnell & Jeanne, 1990; West-Eberhard, 1969) and *Vespula* yellow jackets constitute no exception (Archer 2004; Hurd et al. 2007). On a finer scale, task-specialist workers can exploit specific resources, e.g. visiting only particular flowers or hunting for a subset of prey (Rayor and Munson 2002), paralleling a common pattern among pre-social hymenopterans (Schatz et al. 2001; Polidori et al. 2011; Santoro et al. 2011).

The differential foraging allocation of common wasp workers can be considered behavioural, since size and age could not explain the differences observed between specialists and generalists. Relative to size, we found variation among workers, but there were no significant morphological differences between the two groups. Relative to age, even if specialists were living on average five days longer than generalists, the possibility that associated ontogenetic changes in foraging tasks (polyethism, see Potter 1964) affected the foraging status of the foragers (with “specialists” being “generalists” earlier in their life) could be ruled out, for at least two reasons. First, the workers’ foraging status was consistent when considering only the resources provisioned during e.g. the

first two weeks of life. Second, the proportion of two rarer foraging tasks together (pulp and flesh) was greater in the first two weeks (Santoro, unpublished data) and, theoretically, foragers would be more likely to be “specialists” in the first part of their career.

Our finding that the more active specialist wasps lived longer appears surprising, considering the general trade-off between activity levels and longevity (O'Donnell and Jeanne 1992b; Biesmeijer and Tóth 1998; Wolf and Schmid-Hempel 1989; Robinson et al. 2009). This pattern could be explained by (i) differential costs for the alternative foraging tasks or (ii) benefits arising from individual experience and specialization per se. Indeed, the most common foraging task, fluid collection, took on average longer than other materials' collection, similarly to what was found in other *Vespula* colonies (Archer 2012). Full fluid loads of vespulids are heavier than solid loads (Archer 2012; Kim et al. 2012). Both trip length and load weight can be considered as proxies for foraging costs, and generalist wasp foragers collecting fluids would be hence incurring higher costs. Alternatively, activity-related learning of safe foraging routes and sites or increased handling skills minimizing for example prey subduing risks could explain both the increased efficiency and longer life of task specialists.

An important question remains: what are the proximate causes of the tremendous inter-individual variability observed and what are the ultimate consequences? Although far from a complete understanding, we now know that several intrinsic and extrinsic mechanisms, operating at different levels, can generate inter-individual variation. Within insect colonies, these mechanisms can be genetic, maturational, nutritional, and of environmental nature (reviewed in Jeanson and Weidenmuller 2013). There is evidence that foraging behaviour has a genetic basis in wasps also (O'Donnell 1996), as in honey bees, whose alternative foraging tasks can have a genetic component (Helmich et al. 1985; Robinson and Page 1989).

Research has also emphasized the role of individual experience in shaping task differentiation (e.g. Ravary et al. 2007) and in individual performance

(O'Donnell and Jeanne 1992b). For a wasp, being successful in foraging activities such as hunting can be difficult (Wilson 1971; Spradbery 1973) and requires knowledge and skills that must be learned (Akre et al. 1976; O'Donnell and Jeanne 1992b; Castellanos et al. 2004; Punzo 2005; Slaa and Hughes 2009). Individuals with bolder personalities would be more likely to undertake risk-taking activities such as foraging earlier in their life (Wolf 2007; Biro and Stamp 2008).

Beyond the individual dimension, the colony social environment and its changing needs can drive individual choices (Gordon 1996, Johnson 2010, Santoro 2015). The possibility that colony phenology affected our results on the relationship between specialization and efficiency can be plausibly ruled out, since all the workers included in this analysis were born within 10 days (58 in the same day), and their foraging activity period was largely overlapping. Resource availability, consistency and distribution can also explain different foraging careers between wasps, known for their foraging site fidelity (Raveret Richter 2000; Santoro et al. 2011). Yet, these factors are unlikely to explain the results obtained in such study system, for at least four reasons: (i) Our focal individuals were mostly active during the same days, and as central place foragers sharing the same nest, they could potentially access the same resources (ii) Common wasps show one of the most eclectic diets known, and one flesh load, for example, could be obtained from almost any possible flesh source (Spradbery 1973) (iii) Even if one particular resource was limited, this would in principle affect all foragers (iv) If focal resources were temporally unavailable or spatially clumped, this would affect the behaviour of one individual mostly on short time scales (few consecutive trips), and not over its entire life.

Our data, relative to two colonies of *V. vulgaris*, provide empirical evidence that specialist wasp workers can be more efficient and live longer than generalists. Insect societies are functionally integrated and adaptive units where selection acts both at the individual and at the colony level; hence, any feature that significantly influences colony phenotype and fitness is presumably modulated by selection (Jeanson and Weidenmuller 2013). The cumulative, increased

performance of specialists, even if marginal, can be arguably considered a demonstration of the benefits of individual behavioural specialization for colony fitness. Alternative, additional benefits can arise e.g. from the mitigation of task-switching costs at the colony level (O'Donnell and Jeanne 1990; Jeanson and Lachaud 2015) and from the potential of rare outlier behavioural phenotypes within the workforce, capable of making a difference during unusual events and conditions (O'Donnell & Jeanne 1990; Jeanson and Weidenmüller 2013).

To understand the evolutionary ecology of task allocation, we need further empirical studies focusing on the origins, causes and consequences of individual specialization as well as the link between the degree of inter-individual variability and the colony performance and fitness (Gordon 1996; Jeanson and Weidenmüller 2013). Automated monitoring technologies are now spreading and have an incredible potential in this field. We encourage further studies of this type, hoping in the construction and growth of a broad database where inter-individual differences and task allocation mechanisms can be studied in a comparative approach. At these conditions, theoretical models will find a fertile empirical ground to deepen their roots and strengthen. Indeed, this line of research promises new insights into the evolution of ecological specialism and an important contribution in the general understanding of self-organizing systems, where functioning co-operating units are not centrally organized.

4.6 Supplementary material

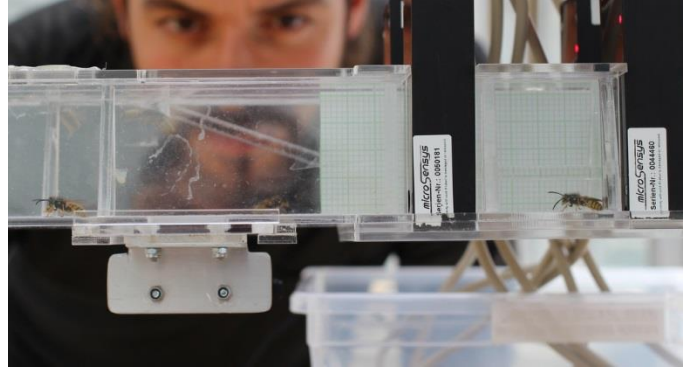


Fig. 4.S1 Wasp nest transparent entrance module with separated lanes for outgoing and incoming wasps, each lane fitting two RFID tunnel readers.

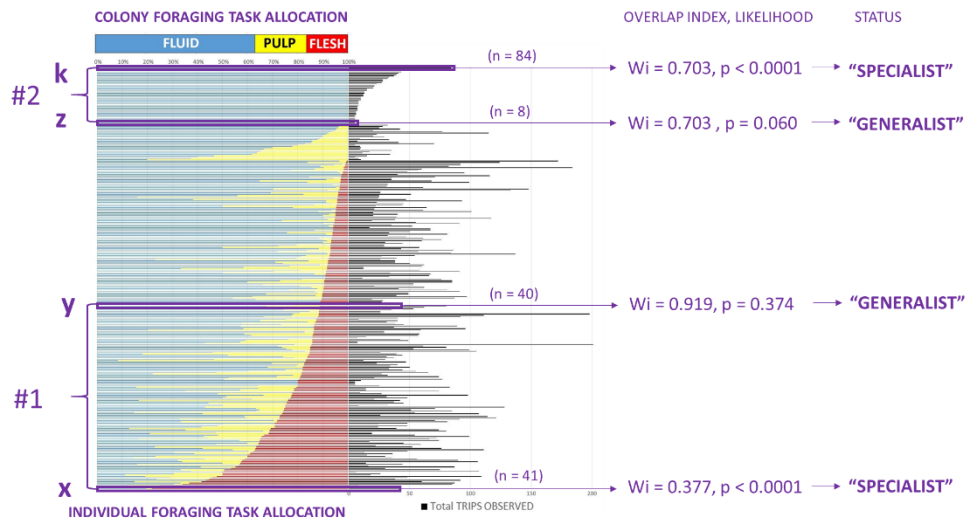


Fig. 4.S2 Worker specialization measured with W_i index, as overlap between individual and colony foraging task allocation. W_i values range from 0 to 1 and the lower the value, the less overlap between the individual and the colony. The p value associated to the W_i value computed by IndSpec1.exe for each wasp measures the likelihood of individual's task allocation being drawn from the task allocation at the colony level. "Specialists" were individuals with $p < 0.05$. With this approach, both the distribution of tasks at the colony level and the consistency in task-specific effort at the individual level are critical to define specialists. Consider one worker (x) dedicating e.g. ca 80% of the trips to a task that is uncommon at the colony level (e.g. flesh foraging): x would have a W_i value way lower than another worker (y) dedicating a comparable number of trips to a prevalent task (e.g. fluid foraging). Worker x would be a "specialist", while y would be a "generalist", because the latter's task allocation largely matches the task allocation at the colony level (1). On the other hand, two individuals (z , k) might show exactly the same task allocation (e.g. exclusively foraging for fluids), hence having the same W_i value, but z be a "generalist" and k a "specialist", in virtue of the greater number of trips performed by the second (2). Given the task allocation at the colony level, any individual performing at least 9 trips and any and one only foraging task would be a "specialist"...

4. Specialized Workers Perform Better

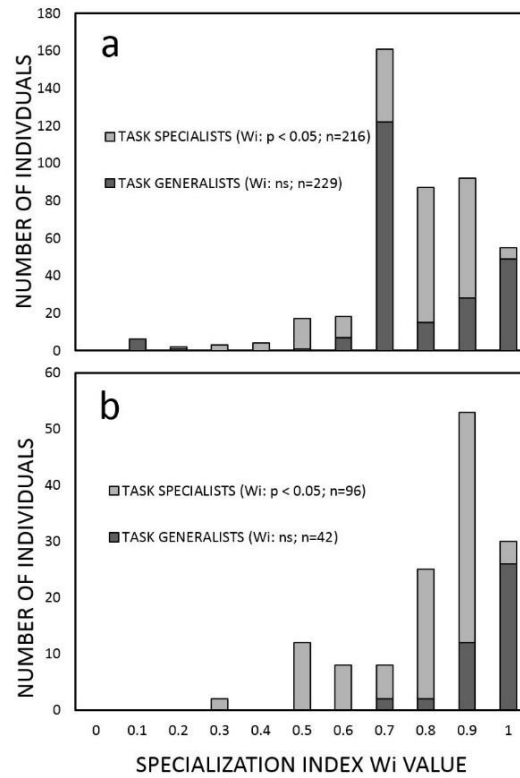


Fig. 4.S3 Distribution of common wasp workers with W_i at or within 0.1 of a given value (e.g. 0.3 = from 0.25 to 0.34). Specialists, individuals showing significant specialization at the $p = 0.05$ level are shown in light grey barring. Generalists are shown in dark grey barring. (a) All individually marked workers observed with loads (b) Individuals with RFID tag included in the lifetime foraging efficiency analysis in the main text.

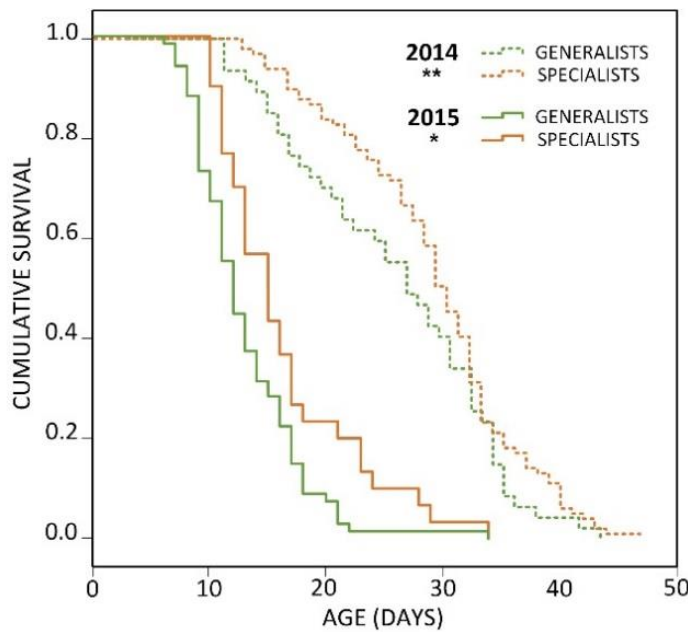


Fig. 4.S4 Survival curves for specialist and generalist wasp foragers from the 2014 (see also main text, Fig. 4.3b) and 2015 colonies (2014: ** $\chi^2_{21} = 8.08, p = 0.005$; 2015 ** $\chi^2_{21} = 6.54, p = 0.011$). Differences in survival between the colonies are likely explained by the death of the queen in 2015, associated to cannibalism and possibly increased worker drifting to other colonies. Differences in weather conditions might have also played an important role.

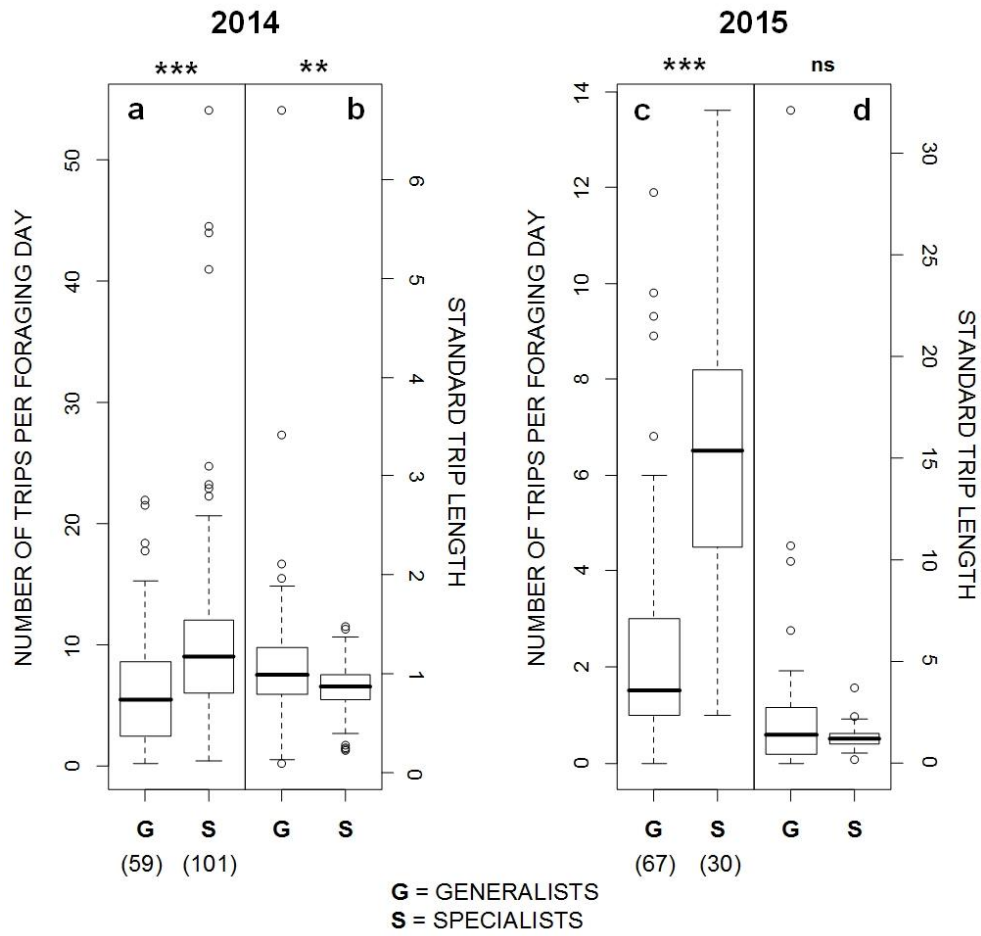


Fig. 4.S5 Individual foraging specialization and performance measured as average number of trips per foraging day (ANTFD) and average standard trip length (ASTL). Boxes represent the lower and upper quartile, the bold line is the median and whiskers represent ANTFD and ASTL extreme values, with the circles identifying outliers. Data from two colonies are showed: The 2014 colony (main text) and the 2015 colony (whose queen was observed dead after one month from the nest setup). Wasps observed at least twice with a load (2 being the minimum number of records allowing specialization in both years) were included in this analysis, and the number of individuals per each group is indicated in parenthesis. (a) *** $\chi^2_1 = 17.60$, $p < 0.001$; (b) ** $\chi^2_1 = 7.40$, $p = 0.007$; (c) *** $\chi^2_1 = 28.91$, $p < 0.001$; (d) ns $\chi^2_1 = 0.43$ $p = 0.512$.

Chapter 5

The Stinging Response of the Common Wasp (*Vespula vulgaris*): Plasticity and Variation in Individual Aggressiveness

5.1 Abstract

We studied the variability and plasticity of individual aggressiveness in a social insect, describing and quantifying the sting extension response (SER) of the common wasp *Vespula vulgaris*. As a proxy for individual aggressiveness we measured the SER of individual wasps, scoring the extent by which the sting was extruded in response to a mild electric shock (7.5 or 12V for 2s) on a scale from 0 to 1. We found that wasps vary greatly in their stinging propensity and aggression thresholds and that individuals change their SER during their life. Extremely aggressive or docile phenotypes, showing at first consistent mutual differences on different days, tended to converge over time and developed comparable SER responses later in their life. Older individuals tended to be more aggressive. Wasp size was not related to the stinging phenotype. Wasp foragers had a less pronounced sting extension than individuals previously involved in nest defense. For the same individual, the aggressive response was proportional to the intensity of the negative stimulus. We discuss the advantages of the SER bioassay as a tool to measure individual aggressiveness, plasticity and inter-individual variability in the Aculeata group, and its great potential in comparative and learning studies.

Keywords Behaviour, inter-individual variability, sting extension, Aculeata, *Apis mellifera*

5.2 Introduction

Social insects represent integrated and adaptive units, the result of selective forces operating at both the individual and colony level (Pinter-Wollman et al. 2012). Phenotypic differences between individuals of the same social insect colony can be striking, and in fact are regarded as a feature of insect societies (Jeanson and Weidenmüller 2013). Indeed, inter-individual variability is acknowledged as a necessary condition for one mainstay of eusociality, the division of labor among colony members (Jeanson and Weidenmüller 2013). The division of labor within social insect colonies has been explained by variability in (i) individual, innate, thresholds and (ii) experience-based, self-reinforcement processes; two non-mutually-exclusive mechanisms (Beshers and Fewell 2001). At the individual level, the role of experience is recognized as central in shaping the individual behavioural phenotype, changing over time via learning processes (Jeanson and Weidenmüller 2013).

Inter-individual differences have been long overlooked in quantitative ecological studies, but have gained new attention during the last decade, notably in the field of “animal personality/behavioural syndrome” research (Violle et al. 2012). A number of studies across the animal kingdom have demonstrated dramatic differences among individual behavioural phenotypes (Bolnick et al. 2003). Since individual phenotypes are the focus of natural selection, any variation at this level has major evolutionary and ecological consequences (Bolnick et al. 2011; Wolf and Weissing 2012).

In biological terms, aggression is a widespread solution by animals to the problems of self-preservation, protection of young and resource competition (Archer 1988). Aggressiveness is a fundamental behavioural and personality trait, varying greatly among individuals (Dingemanse and Wolf 2013; Grinsted et al. 2013). In social hymenopterans, the study of aggressive or defensive behaviour has been classically undertaken in the context of nest-mate recognition and enemy identification (e.g. Guerrieri and D’Ettorre 2008; Breed et al. 2004). For

honey bees and other social Aculeata, one important component of aggressive/defensive behaviour is the act of stinging (Paxton et al. 1994).

The stinging response (stinging reflex, sting extension response) to electric stimulation was described in *Apis mellifera* during the 1980's (Balderrama et al. 1987; Kolmes and Fergusson-Kolmes 1989) and has been used as a bioassay since this time. The sting extension response (SER) was measured via the stimulus intensity (Kolmes and Fergusson-Kolmes 1989; Paxton et al. 1994) or the time necessary to elicit it (Uribe-Rubio et al. 2008). The SER was initially recorded as a binary response (Balderrama et al. 1987; Balderrama et al. 2002), with some authors refining the scoring system by recording the extent to which the sting was extruded, adding intermediate levels of response corresponding to partial sting extension (Nunez et al. 1983; Lenoir et al. 2006). The SER has been quantified to measure honey bees' level of aggressiveness and to discriminate among different strains (Kolmes and Fergusson-Kolmes 1989; Uribe-Rubio 2013), colonies, worker groups (Paxton et al. 1994; Uribe-Rubio 2013) and individuals from the same hive (Lenoir et al. 2006). Variation in SER was found in relation to honey bees' age and within-colony social environment (Paxton et al. 1994), spatial segregation in the hive (Uribe-Rubio et al. 2008), task performed (Uribe-Rubio et al. 2008, 2013), and paternity line (Lenoir et al. 2006).

It is important to highlight that, depending on the organism under examination, the same type of behavioural response (e.g. SER) can have a radically different meaning in biological terms. Honey bees sting for defense, and although they can sting other insects without harming themselves, their sting is strongly barbed and remains embedded in mammalian flesh (Vetter et al. 1999). Given the characteristic sting autotomy, anchoring and the consequent abdominal rupture, honey bees' stinging behaviour can represent an extreme form of suicidal colony defense (Paxton et al. 1994; Shorter and Rueppel 2012). Relatively little is known about the stinging behaviour of other eusocial bees (reviewed in Breed et al. 2004), and we are not aware of any studies quantifying the stinging response in wasps, using their sting as a defensive and offensive weapon.

Given their sting morphology, social wasps are usually able to sting multiple times (Vetter et al. 1999), although sting anchoring routinely occurs in some species such as *V. maculifrons* (Greene 1991).

For wasps, aggression is a common practice, e.g. in foraging activities (Parrish 1984; Raveret Richter 2000), social dynamics and in high risk situations (Ross and Matthews 1991). The majority of aculeate wasps are solitary and use their stings to paralyze hosts more or less permanently and then carry them to a shelter where an egg is laid on the host (Raveret Richter 2000). For social aculeate wasps, prey are generally killed with the characteristically strong mandibles, rather than stinging (Edwards 1980; Raveret Richter 2000). Nevertheless, several authors (reviewed in Spradbery 1973; Olson 2000) documented cases in which foraging social wasps used their sting when grappling with particularly large and active prey. *Vespa orientalis* was reported to make regular use of the sting when attacking *A. mellifera* in Israel (Ishay et al. 1967, cited in Spradbery 1973). This evidence contradicts some statements, that social wasps kill their prey using exclusively their mandibles (e.g. Akre and Myhre 1992, in Olson 2000; Archer 2012).

Social wasps also utilize their stings during frequent inter- and intra-specific conflicts among gynes, workers and castes (Archer 2012). During spring, mortal fights between queens competing for nesting sites are the norm (Greene 1991; Matsuura and Yamane 1990; Matsuura 1991; Hunt 2007). In some yellow-jackets (e.g. *Vespula*, *Dolichovespula*), usurpation may be more common than nest initiation (Greene 1991), and disputes appear more frequent among conspecifics (Buck et al. 2008). The sting can also be used by workers to kill the males (Monceau et al. 2013), or during matricide when workers kill their queen (Loope 2014). In general, aggression (e.g. mauling, biting and grappling) among workers is common, both at the foraging sites (Parrish 1984; Raveret Richter 2000) and inside the nest (Akre et al. 1976; O'Donnell and Jeanne 1995). Fighting, including attempted stinging, usually involves workers with developed ovaries, both in colonies with and without a queen (Archer 2012).

Stinging is the immediate response in a life-threatening situation, both for the individual wasps and the colonies. Intruders inside a social nest are stung in rapid actions, involving one or more co-operating wasps (Edwards 1980). If the nest is disturbed from outside, agitated workers rush out and eventually take flight, with a minority of individuals normally stinging the target (Greene 1991). In species with large colonies, active nest defense can involve hundreds of individuals and mass stinging can potentially be lethal to humans (Vetter et al. 1999). Vespine wasps, including yellowjackets, are particularly aggressive and are the group usually responsible for hymenopteran stinging events involving humans (Vetter et al. 1999). Wasps defend their nests fiercely (Edwards 1980) and may also sting disturbers while foraging, especially in the late summer and early fall, when many hungry queen larvae must be fed with flesh (Spradbery 1973).

Because of their aggressiveness, predatory habits and sting features, workers of social wasps have the potential to sting more than once throughout their life, in a variety of contexts. As a consequence, it is possible that the stinging phenotype of one individual may be shaped by previous experience. As highlighted in the past, there are a number of unresolved problems with the study of wasps' stinging behaviour (Greene 1991; Olson 2000). In general, the descriptions of specific aggressiveness for wasps are mostly anecdotal, qualitative and contradictory. Knowledge of the factors regulating individual wasp response thresholds for various disturbing stimuli is very limited. Alarm pheromones, worker age, colony size, brood population, previous disturbance, and meteorological conditions represent some commonly discussed factors influencing the stinging behaviour (Gaul 1953; Balduf 1954, cited in Ross and Matthews 1991; Potter 1964; London and Jeanne 1996; Reed and Landolt 2000, London and Jeanne 2003).

In the present paper we quantify individual wasps' stinging propensity, with a laboratory bioassay. As a proxy for aggressiveness, we describe and measure the sting extension of the common wasp, *V. vulgaris*, looking at its individual plasticity and inter-individual variation. Taking into account individual age and

size, we measured variation in individual SER throughout the adult life of these wasps, relating it to previous experience, presumptive task performed by the tested individuals and intensity of noxious stimulation.

5.3 Materials and methods

5.3.1 Wasp colonies and study site

A *V. vulgaris* nest (Colony 1) was excavated in Christchurch, New Zealand, on the 19th of January 2014. The nest was boxed and carried to a greenhouse facility at the Plant & Food Research Biosecurity laboratory, Lincoln. Wasps were allowed to forage outside of the nest box, in the grounds surrounding the laboratory. On the 18th of March 2014, the colony was anesthetized with carbon dioxide, the box opened, and three pieces of nest combs with capped brood were removed and placed in an incubator (30°C, 50% relative humidity, and complete darkness). A total of 292 known-age workers were obtained between the 19th of March and the 7th of April 2014. Newly emerged adults were collected daily, caged and tested for their SER. The cages were 50 ml plastic jars modified by covering either end with a metal mesh. The individually-caged wasps were fed daily by imbibing with 1 ml of 30% sugar solution one cotton bud suspended from the lid mesh. The cages were numbered and organized on trays lined with absorbent paper that was changed daily, and kept in the incubator under the same conditions as the nest combs. Two additional *V. vulgaris* nests were located in the grounds of the research facility at Lincoln. Exiting wasps from these colonies (Colony 2, 3) were collected and tested, to investigate the relationship between SER and stimulus intensity (see below, 5.3.6).

5.3.2 SER scoring

The caged, known-age individuals were lightly anesthetized with carbon dioxide and, when subdued but still moving, harnessed by holding their petiole from the side with metal forceps (Bioquip, Feather Weight Forceps, Narrow

Tip), kept closed with one clip (Fig. 5.1, Fig. 5.S1). Each individual was allowed 30 minutes to acclimate to the harnessing before testing. The forceps restraining the wasp were connected to one polarized cable, while the other one was connected to a metal arch covered in conductive gel (Spectra electrode gel, contents 8.5 OZ, 250GMS, salt free, Parker laboratories Inc.) (Fig. 5.S2). The electric stimulation was delivered for two seconds by touching the wasp's cervix with the metal arch. The standard stimulus intensity was 7.5 Volts (Balderrama et al. 2002). Each wasp was always tested four consecutive times (rounds), with an inter-round interval of one minute. Responses during each stimulation were recorded by observing the tested individuals' abdomen from the side, adopting the four-levels scoring system proposed by Lenoir et al. (2006) for honey bees. The absence of any response was scored 0, the response was scored 0.33 when the sting was extended less than the half of its length, 0.67 when it was extended between half and all its length and finally, the response was scored 1 when the sting was fully extended and the sting chamber was completely open. The wasps' final SER score was obtained by averaging the four consecutive measures and hence varied between 0 (minimum aggression response) and 1 (maximum aggression response) (Fig. 5.S3). Student's pairwise t-tests were used to compare the individual SER between the first trial's rounds.

5.3.3 SER in relation to individual age and size

A total of 264 known-age individuals were tested for the first time at different ages (1-27 days of adult life). A subset of 112 wasps were preserved in 90% ethanol at -20°C, and their head width was subsequently measured using a digital calliper to the nearest 0.01 mm. To separately test for an effect of age (using all the known-age individuals) and size on the SER score, simple linear regression was used. For the individuals of known age and size, we also performed a multiple regression testing the effect of both variables on the SER score.

5.3.4 Plasticity in individual SER throughout life

One subset of 122 known-age wasps was kept in individual cages (see above) and the individuals were retested at 48 h intervals (trials) throughout their remaining life. A total of 112 individuals were tested at least twice (1st test age: 10.0 ± 5.5 , 1-19 days). Their average adult lifespan was 18.1 ± 5.1 , with a range of 8-28 days. On the base of the initial SER score at day 1, two subgroups were distinguished: 31 individuals had a SER score = 0 for the first trial (no sting extension response on the four consecutive rounds). Of these, 26 individuals could be retested and were pooled in the “docile group” (1st test age: 9.4 ± 4.2 , 1-13 days). The 29 individuals showing the highest SER score on the first trial (= 0.83) were assigned to the “aggressive group” (1st test age: 8.1 ± 5.7 , 1-16 days). To analyse the change of the individual SER during lifetime, repeated measures analysis of variance with Bonferroni confidence interval adjustment was performed in pairwise comparisons among trials (days). Since data were not normally distributed, Kruskal-Wallis test was used to compare the SER score of the docile group vs aggressive group, trial by trial.

5.3.5 SER and presumptive task (forager/guard)

Two task-related groups of wasps were sampled from Colony 1. Over three days, we captured 52 incoming foragers with a butterfly net, while flying with their foraging loads in proximity of the nest. On the same days, after the capturing the foragers, we hit the substrate supporting the nest with a stick, provoking a colony defense reaction, and captured a total of 45 “guards” that emerged from the nest. Wasps from both groups were transferred in individual cages, and their SER examined within 2h. Since data were not normally distributed, Kruskal-Wallis test was used to test for differences in the SER between the groups of foragers and guards.

5.3.6 SER and stimulus intensity

Individuals exiting the three colonies were captured and then tested at 7.5 and 12.5 Volts (Colony 1: 78 individuals; Colony 2: 26 individuals; Colony 3: 12 individuals). For each individual, the inter-test interval was set to 30 minutes and the voltage order established randomly. Student's pairwise t-test was used to compare the individual SER between the two tests. All data analysis was performed using R 3.1.0 (R Development Core Team 2013) and IBM SPSS Statistics 21 (IBM Corp. 2012).

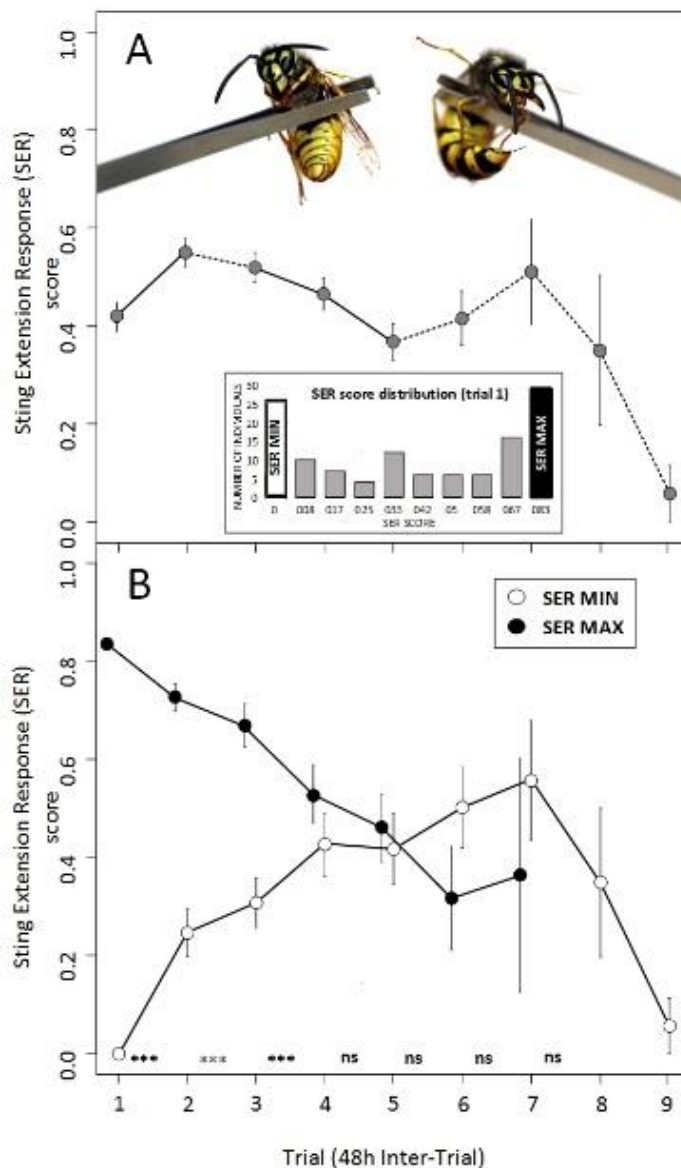


Fig. 5.1 A. Individual plasticity of the sting extension response (SER, circles) throughout life, on the basis of previous noxious experience. Points are mean \pm SE. Continuous lines indicate significant differences between consecutive trials. The pictures represent phenotypes at the two extremes (docile vs aggressive). The inset histogram in the box shows the distribution of all the first trial's SER scores ($n=122$) and the two subgroups (white and black bar). B. Individual SER plasticity for the initially aggressive group (SER MAX, Black points, individuals with the highest stinging score recorded on the first trial) and the initially docile group (SER MIN, White points, individuals showing no response on the first day trial). Trials were separated by approximately 48 hours. Points are mean \pm SE. P values refer to difference in SER score between aggressive and docile group, tested trial by trial. *** = $p < 0.001$, ns = $p > 0.05$. Please refer to Table 5.S2 (supplementary material), for details of the statistical comparisons.

5.4 Results

5.4.1 SER in relation to age and size

Substantial and consistent variation in individual stinging response was found between wasps (average SER score \pm SD: 0.64 ± 0.40 ; $n = 292$). On the first trial, one individual out of five showed no response to the stimulation and half of the wasps showed the full sting extension on at least one round. Within each trial, the individual SER score was consistent across rounds (Table 5.S1). Only starting from the second trial, five individuals (one for the next two) scored 1 (full sting extension on the four consecutive rounds). Despite wide variability in SER scores, a positive relationship between age and SER was found (Multiple $R^2 = 0.066$, $R^2_{\text{adj}} = 0.062$, $F_{1,262} = 18.45$, $p < 0.0001$) (Fig. 5.2). When both age and size were considered, for a subset of 105 individuals, no significant effect of the two factors was found (effect of age: $F_{1,102} = 1.35$, $p = 0.251$; effect of size: $F_{1,102} = 0.09$, $p = 0.765$. Model $R^2 = 0.013$, $R^2_{\text{adj}} = -0.006$).

Individuals varied considerably in size (3.08 – 3.71 mm head width). However, no relationship was found between individual size and SER score (Multiple $R^2 = 0.0024$, $R^2_{\text{adj}} = -0.007$, $F_{1,110} = 0.264$, $p = 0.608$) (Fig. 5.3).

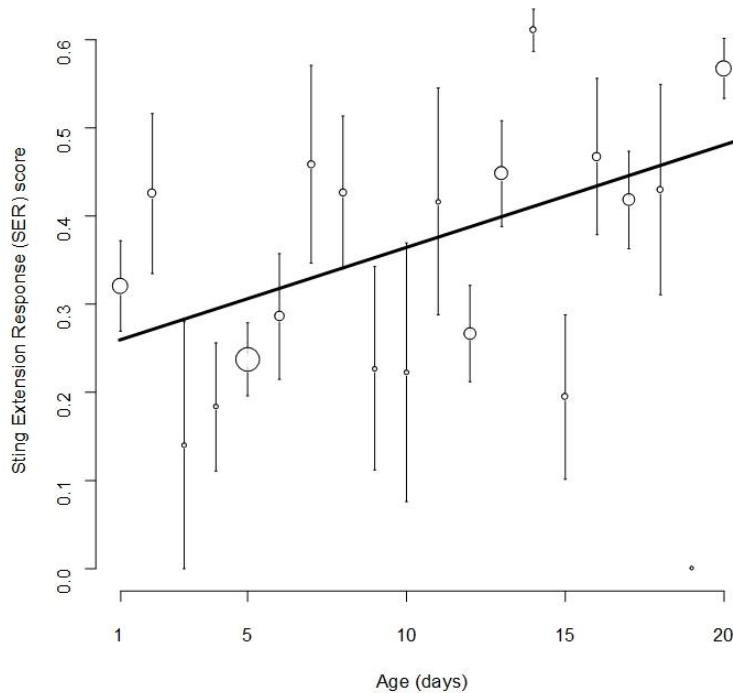


Fig. 5.2 Regression between the sting extension response (SER) and the age of the wasp. Point symbols are mean \pm SE. The point's diameter is proportional to the sample size of each age (total $n = 264$, n per day = 2-47).

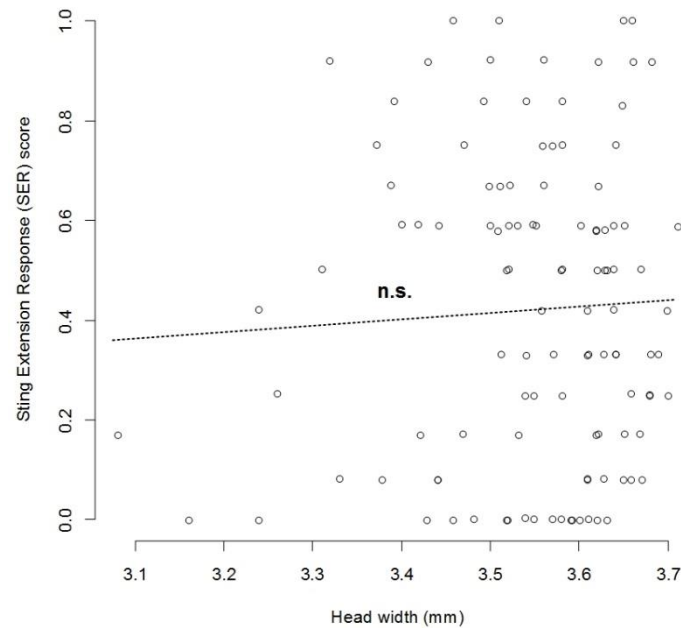


Fig. 5.3 Correlation between the sting extension response (SER) and the size of the wasp. Each point represent one individual and its SER score at the first test.

5.4.2 Individual SER plasticity throughout life

There was a significant change in individual SER over successive trials and days. Overall, the average stinging response was stronger on the second trial and subsequently decreased (Fig. 5.1a). For the group of individuals retested, the distribution of SER on the first trial was bimodal, with 21% of individuals displaying a SER score of 0 (pooled in the “docile” group) and 24% showing a SER score of 0.83 (pooled in the “aggressive” group) (Fig. 5.1a (inset)). The docile and aggressive groups showed opposite trends over time. The aggressive group displayed a significantly higher SER score than the docile group for the first three trials, but with a decline in SER over time, while the docile group showed an increase in SER over the first seven trials. The two groups showed a similar score from the fourth to the seventh trial (Fig. 5.1b, Table 5.S2). Some individuals within these groups never changed, always displaying very low or very high SER scores.

5.4.3 SER and presumptive task (forager/guard)

On average, the SER score of presumptive guards was three-fold higher than the SER score of the foragers ($\chi^2 = 19.54$, $df = 1$, $p < 0.0001$). Nevertheless, inter-individual variation within the two groups was wide (Fig. 5.4).

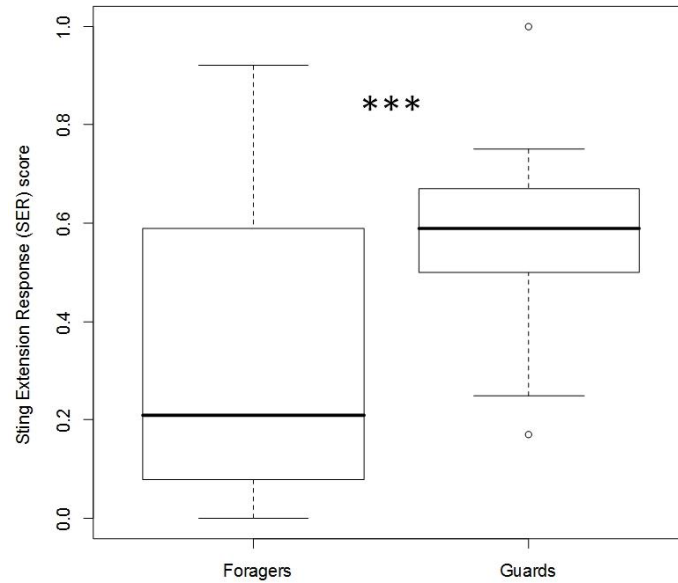


Fig. 5.4 Sting extension response (SER) and presumptive task undertaken by tested individuals. “Foragers” were captured on their way back to the nest. “Guards” were collected after striking the nest box. Boxes represent the lower and upper quartile, the bold line is the median and whiskers represent extreme values of SER score, with the circles identifying outliers. *** = $p < 0.001$.

5.4.4 SER and stimulus intensity

Wasps from all the three colonies showed a significantly stronger stinging response in correspondence to higher stimulus intensity. The SER score at 12.5 V was up to almost four times higher than at 7.5 V (Colony 1: $t = -13.70$, $df = 77$, $p < 0.0001$; Colony 2: $t = -7.84$, $df = 25$, $p < 0.0001$; Colony 3: $t = -3.06$, $df = 11$, $p = 0.0110$). Some individuals showed no or little response at both stimulus intensity levels (Fig. 5.5).

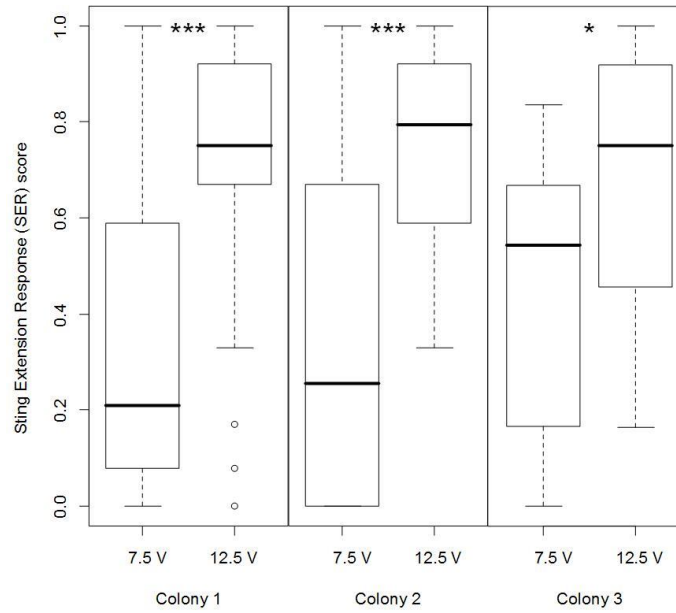


Fig. 5.5 Sting extension response (SER) of individuals from three *Vespula vulgaris* colonies, tested twice at different intensities of noxious stimulus (electric current), in random order. Boxes represent the lower and upper quartile, the bold line is the median and whiskers represent extreme values of SER score, with the circles identifying outliers. *** = $p < 0.001$, * = $p < 0.05$.

5.5 Discussion

Given the ubiquitous occurrence of direct competitive interactions, aggression plays a major role in fitness outcomes (Archer 1988). Despite being reported numerous times in many contexts, differences in defensiveness/aggressiveness among social hymenopteran species (e.g. Breed 1994; Greene 1991), between and within specific colonies (e.g. Jeanne 1988; Pinter-Wollman 2012) have been described mostly qualitatively. Scoring the sting extension response, as a proxy for aggressiveness, can quantify an important trait of the individual behavioural phenotype (Dingemanse and Wolf 2013; Grinsted et al. 2013). The SER bioassay hence represents a potential tool for the study of inter-individual variation and animal personalities in an evolutionary perspective. A growing body of literature is showing the importance to ecological studies of quantifying individual variability (Bolnick et al. 2003; Bolnick et al. 2011; Violle et al. 2012; Wolf and Weissing 2012). We believe that the sting extension response is an

opportunity to study behavioural variability among the social Aculeata, both at an individual level and in comparative studies.

We tested for, and found, substantial and consistent variation in the stinging thresholds of common wasp nestmates. Although if data for Colony 2 and 3 are very limited, this behavioural pattern was consistent for all wasp colonies examined. The individual SER measure proved to be repeatable across rounds and trials (Fig. 5.1b, Table 5.S1, 5.S2). The plasticity of SER recorded throughout a wasp's life highlights the variability in time of individual behaviour. Initially docile wasps developed a stronger SER, while initially aggressive wasps showed an opposite trend, almost having a lower SER score compared to the first group later in their life (Trial 6: Fig. 5.1b, Table 5.S2b). These inverse patterns seem to exclude the possibility of a simple sensitization to the electric stimulation (Shettleworth 2010). Also, artificial, asocial and stressful captivity conditions would likely affect individuals similarly. Our data do not allow to speculate on the significance of these plastic changes in a natural context, but still show that extremely different phenotypes can converge and express similarly, on the base of the same previous experience. Taken together, our empirical findings support theoretical frameworks where individuals show innate differences but change their behavioural phenotype throughout their life (Theraulaz et al. 1998; Beshers and Fewell 2001).

Age was correlated to the stinging propensity of *V. vulgaris* individuals. This result finds a parallel in field-based studies performed on other social wasp species, where older individuals were found to be more likely to defend the colony (Jeanne et al. 1992; Togni and Giannotti 2010; Monceau et al. 2013). However, when including head width as a covariate, no relationship between age and SER was found, probably due to the high intra-trial variability and reduction in sample size.

It must be noticed that age poorly explained the variation observed. Moreover, the isolation of known age adults might have influenced their behavioural phenotype. The stinging reaction was observed in individuals of all ages, including

in one-day-old individuals. Ontogenetic variation in stinging behaviour has been reported for honey bees, with older individuals (20 days old) showing the lowest stinging thresholds (Paxton et al. 1994). The sting reflex was found to be fully developed in bees older than 5-7 days (Breed et al. 2004). The median age at which *A. mellifera* workers become guards was reported to be 12 days (Lenoir 2006). Older individuals have lower life expectancy and theoretically are predicted to be more likely to perform risky tasks, e.g. nest defense, their anticipated future value to their society being lower (Kolmes and Fergusson-Kolmes 1989).

We found no significant relationship among individual size and SER. Still, it should be noted that small individuals with head width < 3.3 mm never displayed a SER score greater than 0.5 (Fig. 5.3), perhaps suggesting a non-linear relationship. To our knowledge, no study has so far investigated a possible relationship between sting extension and individual size. *Vespula* spp. colonies are good systems, being characterized by seasonal morphometric changes of workers, with increasing average worker size and variation as the colony develops (Spradbery 1972). Size relationships represent a crucial factor in most predator-prey and intra-specific competitive interactions (e.g. MacNulty et al. 2009; Santoro et al. 2011). Together with age, size is also extremely important in establishing dominance hierarchies in social insects (O'Donnell and Jeanne 1995; Hogendoorn and Velthuis 1999). Dominance relationship among colony members can, in turn, influence the division of labor among and within castes (O'Donnell and Jeanne 1995). Size was also shown to directly determine individual behavioural thresholds: bigger bumble bees (*Bombus terrestris*), for example, are more likely to forage and have more sensitive antennae, showing lower thresholds to odours (Spaethe et al. 2007).

Despite their intrinsic limitations, measures obtained in solitary, standardized laboratory settings can be reliable predictors of behaviour in a social context in the field (Grinsted et al. 2013). The SER bioassay detected wide variability in stinging propensity and discriminated individuals previously involved in colony defense from foragers. These results match field observations, that stinging and

nest active defense by social hymenopterans involves only a subset of workers (Greene 1991; Breed et al. 2004). For example, Africanized honey bee guards were faster to sting than nest bees (Uribe-Rubio et al. 2008). Given that our wasp “guards” were tested within two hours from the colony provocation, they might have still been under the effect of an alarm pheromone, for which the composition and latency are still to be investigated (Reed and Landolt 2000).

The stinging response of individuals from the three colonies was more pronounced in association with a more intense electric stimulation. The same individual showed a more aggressive reaction to the stronger of the two noxious stimuli, regardless of which one was experienced first. Similar results were obtained with different groups of honey bee workers, whose responsiveness to electric stimulation was found to increase with higher voltages, up to 8 V (Balderrama et al. 2002).

Variation in SER can have a genetic basis. A number of studies on *A. mellifera* have demonstrated that guarding is a specialized task performed by a few, genetically predisposed individuals (Robinson 1992; Breed et al. 2004; Hunt 2007). Inter-individual variability in the honey bee SER has been observed as associated with different paternity lines (Lenoir et al. 2006). Polyandry is rare among social hymenopterans (Strassmann 2001) and, despite having costs in terms of colony cohesion (Crozier and Fjerdingstad 2001), was linked to colony fitness in e.g. *V. maculifrons* (Goodisman et al. 2007). Polyandry is thought to have an adaptive significance by tuning group responses of insect colonies, determining worker subgroups with different task-related thresholds (Oldroyd and Fewell 2007). Both *A. mellifera* and *Vespula* are peculiar amongst hymenopterans, showing marked polyandry (Strassmann 2001, Hana et al. 2013). Paternal effects in wasp’s aggressiveness await to be tested. However, we predict that colonies and species with single-mating queens will show lower levels of aggressive variability among nestmates, measurable with the SER bioassay.

A very promising avenue is the SER classical conditioning for research focusing on the differential learning performances among Aculeata. Successful

aversive conditioning was done on *A. mellifera* (e.g. Vergoz et al. 2007). Wasps are traditionally described as explorative, opportunistic foragers, capable of quick associative learning (Raveret Richter 2000; Moreyra et al. 2014). Wasps' associative learning capabilities await to be scored in the laboratory.

Our findings add to the existing literature by extending earlier SER studies on *A. mellifera* to a new system, vespine wasps. We quantified the degree individuals from the same insect colony can differ in the SER, providing an example of experience-based convergence of initially different behavioural phenotypes. The quantification of the stinging behaviour through the SER score proved to be a repeatable, easily-performed procedure to test for innate threshold differences between individual wasps. We encourage the development of standardized SER testing methods and more studies to better understand the biological significance of SER, focusing on the relationship between the measures obtained with this laboratory bioassay and aggressive/defensive interactions in different natural contexts. At these conditions, the SER bioassay have the potential to become a standard measure of aggressiveness, a cardinal personality trait. Even more interestingly, the SER comes with the promise of exciting advancement in comparative and learning studies.

5.6 Supplementary material



Fig. 5.S1 *Harnessed Vespula vulgaris worker.*



Fig. 5.S2 *Vespula vulgaris worker ready for sting extension response (SER) test.*

5. The Stinging Response of the Common Wasp

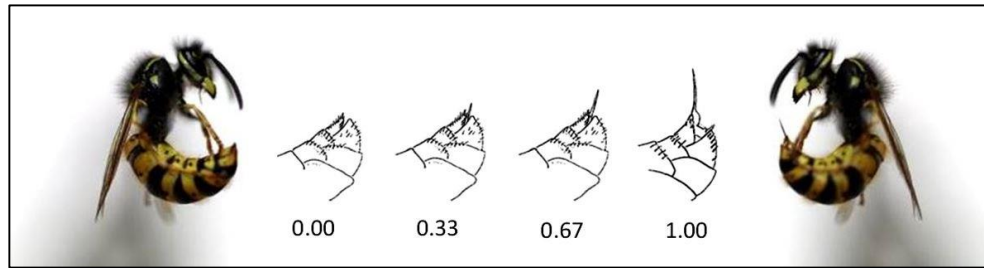


Fig. 5.S3 *Sting extension response (SER) scoring. On each trial, wasps were tested for four consecutive times (rounds). The SER score was obtained by averaging the response on the four rounds. Figure modified from Lenoir et al. 2006.*

Table 5.S1 *Individual SER consistency accross rounds, within the first SER trial. Known-age individuals kept in the laboratory and workers taken directly from the three colonies are pooled together.*

ROUND	I	II	III	IV
SER average \pm SD	0.47 \pm 0.35	0.47 \pm 0.36	0.47 \pm 0.36	0.49 \pm 0.37
ROUND vs ROUND	I-II	II-III	III-IV	
t	-0.54	-0.053	-1.135	
df	476	476	476	
Sig.	0.589	0.958	0.257	

Table 5.S2 *A. Statistical comparisons (repeated measures analysis of variance with Bonferroni confidence interval adjustment) among trials for all individuals tested multiple times (ALL) and for docile (SER MIN) and aggressive (SER MAX) subgroups. B. Trial by trial comparisons (Kruskal-Wallis test) between docile (SER MIN) and aggressive (SER MAX) subgroups. Significant differences are in bold.*

A			1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9
GROUP / TRIAL										
ALL	Sample size		112	105	87	64	32	12	6	3
	Sig.		0.000	0.365	0.001	0.014	0.827	0.565	0.381	0.284
	Wilks' Lambda		0.849	0.992	0.882	0.907	0.998	0.969	0.844	0.487
	F		19.676	0.828	11.494	6.423	0.049	0.352	0.921	2.109
	Partial Eta Squared		0.151	0.008	0.118	0.093	0.002	0.565	0.156	0.513
SER MIN	Sample size		26	23	22	19	14	9	6	3
	Sig.		0.000	0.210	0.204	0.983	0.870	0.673	0.381	0.284
	Wilks' Lambda		0.592	0.931	0.924	1.000	0.998	0.977	0.844	0.487
	F		17.244	1.624	1.722	0.000	0.028	0.192	0.921	2.109
	Partial Eta Squared		0.408	0.069	0.076	0.000	0.002	0.023	0.156	0.513
SER MAX	Sample size		28	27	25	19	9	3	0	0
	Sig.		0.001	0.472	0.010	0.483	0.074	0.214		
	Wilks' Lambda		0.667	0.980	0.752	0.972	0.615	0.382		
	F		13.451	0.532	7.911	0.513	4.388	3.238		
	Partial Eta Squared		0.333	0.020	0.248	0.028	0.385	0.618		
B			1	2	3	4	5	6	7	
SER MIN vs SER MAX / TRIAL										
SER MIN sample size			26	26	23	22	19	14	9	
SER MAX sample size			29	28	27	25	19	8	3	
chi-squared			58.000	31.357	18.744	0.890	0.167	2.595	0.551	
df			1	1	1	1	1	1	1	
Sig.			2.62E-14	2.15E-08	1.50E-05	0.346	0.683	0.107	0.458	

Chapter 6

General Discussion

6.1 Thesis summary

Social insects are among the most abundant, diverse and widespread taxonomic groups. Their ecological success is frequently attributed to division of labour, worker specialization and workforce co-ordination within colonies (Wilson 1971; Chittka and Muller 2009). Despite the intimidating amount of literature available on social insects behavioural ecology, little studied is behaviour at the level of the individual, and far from being understood are the proximate causes and consequences of division of labour (Dornhaus 2008; Chittka and Muller 2009; Jeanson and Weidenmüller 2013). Social wasps (Hymenoptera: Vespidae) have a recognized role in teaching us about social evolution, for they allow comparative studies among species showing all stages of sociality, from solitary to eusocial. Yet, our knowledge of very basic aspects of social wasps' biology (such as information transfer mechanisms, workforce organization, colony defence or queen-control mechanisms) is still very poor. With my thesis, I aimed to contribute to a better understanding of the common wasp (*Vespula vulgaris*) foraging ecology and organization of labour. I investigated the variability of behaviour between wasp colony workers and their cooperation.

My research suggests that foraging in common wasps is mostly an individual endeavor, but there appears to be evidence for information sharing and co-ordination in foragers' activity. In fact, the discovery and choice of resources by wasp foragers was assisted by information provided by experienced nestmates (Chapter 2). Information sharing was relative to chemical cues associated with the food and possibly to its location. Moreover, when resources known to portion of the workforce became newly available, the foraging effort of the whole colony increased. These data are consistent with foraging activation mechanisms. My observations of common wasps at the feeding stations suggest piloting (in which one individual leads one or more nestmates to a resource (Aguilar et al. 2005)) as a possible foraging recruitment mechanism in social wasps.

I found huge variation in activity, task performance, and survival among *V. vulgaris* individuals (Chapter 3). Some workers specialized on alternative foraging tasks over their lifetime, and some individuals performed a disproportionately high number of foraging trips. Both individual age and size played a role in the foraging behaviour. Foragers appeared to become more successful with age, performing more trips and carrying heavier fluid loads. Compared to smaller nestmates, larger wasp workers contributed more to the colony foraging economies. High mortality rates were associated with the beginning of the foraging activity, relative to lower mortality in more experienced workers.

I found empirical support for the widespread but rarely tested hypothesis that specialist foragers are more efficient than generalists, evaluating the performance of wasp workers within the same insect colony (Chapter 4). In fact, *V. vulgaris* behavioural specialists performed more trips per foraging day and their trips were relatively shorter. Despite their more intense foraging effort, specialists lived longer than generalists.

I investigated the intra-colonial variation in aggression thresholds of common wasps. As a proxy for individual aggressiveness, I developed and described the sting extension response (SER) of individual wasps responding to a mild electric shock (Chapter 5). I found that wasps vary greatly in their stinging propensity and that individuals change their SER during their life. Extremely aggressive or docile phenotypes, showing at first consistent mutual differences on different days, tended to converge over time and developed comparable SER responses later in their life. Older individuals tended to be more aggressive. Wasp size was not significantly related to the stinging phenotype. Wasp foragers had a less pronounced sting extension than individuals previously involved in nest defense. For the same individual, the aggressive response was proportional to the intensity of the negative stimulus.

6.2 Synthesis with wider literature

To date, there has been no clear evidence that social wasps actively recruit nestmates to food sources (Raveret Richter 2000; Jeanne and Taylor 2009). Various hypothesis have been formulated to explain this apparent lack of communication within colonies, setting social wasps apart from other hymenopterans. Possible constraints proposed are of social and ecological nature, and include colony size, distribution of exploited resources, habitat type, genetic variability, and food storage inability (Jeanne et al. 1995; Dornhaus and Chittka 2004a; Hrncir et al. 2007). Yet, my observations on *V. vulgaris* foragers are consistent with location-specific communication among nestmates, suggesting pilot flights as a possible recruitment mechanism. Together with previous research made on other Vespidae (Matsuura & Yamane, 1990; Naumann, 1970; O'Donnell & Hunt, 2013; Taylor, 2012), my findings suggest that further research will reveal currently unknown communication mechanisms within this group of social insects. The evidence found for nest-based information transfer and foraging activation in the common wasp further highlights the convergent evolution of food recruitment mechanisms within hymenopterans (Nieh 2009), and suggests an additional mechanism for their widespread success (Lester et al. 2014).

Particularly during the last decade, a growing body of literature has been highlighting the importance of intra-specific variation in the ecology and evolution of animal species (Bolnick et al. 2003; Wolf et al. 2007; Violle et al. 2012). The differences between conspecifics can exceed those with heterospecific individuals and can have major fitness consequences. Investigating the causes of inter-individual variation within insect societies faces the difficulty of understanding whether the patterns observed result from selection at the individual and/or at the colony level. On the other hand, studying inter-individual variability in insect societies is particularly important, because it represents a main avenue of understanding one of their key features: the division of labour. I found evidence that specialist foragers are more efficient than their nestmates, and this

empirical finding supports the view that inter-individual variation can have an adaptive role at the colony level.

In a range of different contexts, colonies could greatly benefit from the presence of individuals extremely bold and explorative or aggressive, which could become “elite” in crucial and risky activities such as foraging or nest defense. Rare behavioural phenotypes within the workforce have been hypothesized to be fundamental for the colony during unusual events and conditions (O'Donnell and Jeanne 1990; Jeanson and Weidenmüller 2013). Given the potential fitness advantages of societies including individual outliers, extreme variation among individuals and exceptional phenotypes might be particularly favored within social species, even if selected traits are deleterious at the individual level.

The phenomenon of elitism (the presence of a small proportion of individual workers engaged in a task and performing a disproportionate amount of the work achieved by the colony as a whole) has been observed in bees, wasps (this study on the group being the first one to our knowledge utilizing RFID technology) and ants (Hurd et al. 2003; Robinson et al. 2009; Tenczar et al. 2014). Elitism could be alternatively explained by (i) the presence of a distinct subcaste of intrinsically particular workers (Hurd et al. 2003) (ii) an extreme of a range of activity levels (Tenczar et al. 2014), depending on extrinsic factors such as resource availability (Beverly et al. 2009). On the other extreme of the activity spectrum, “lazy” workers could be colony reservoirs (Charbonneau and Dornhaus 2015) or social opportunists adopting alternative strategies aimed to maximize their individual fitness directly, being ready in events such as the queen's death (Strassmann et al. 2003).

6.3 Constraints and problems encountered

Much of the behavioural data available on social wasps, particularly Vespinae, come from the study of a limited number of species and replicates. The present research and our knowledge on this group of wasps has been constrained by the challenges imposed by their specific ecology. The study of Vespinae is difficult. In fact, the activity of a vespulid colonies is concentrated in a relatively narrow time window. Moreover, *Vespula* queens start their nests alone and early colonies are small, fragile and difficult to locate (Donovan 1991). Within the course of this thesis research, my attempts to establish laboratory colonies with overwintering queens failed, confirming the difficulties encountered by previous researchers using indoor rearing units (Ross et al. 1981; Leathwick 1997). The limited time available on each summer and the RFID system used allowed the study of only one colony per year. It was not possible following the activity of completely non-overlapping generations of workers. The nest studied during summer 2014 grew in size and allowed a second extraction of capped brood combs after the death of the last RFID-tagged workers. Yet, it was already late in the season, weather conditions were worsening and after the new manipulation the colony showed signs of distress (cannibalism set in, part of the workforce started a new nest structure outside of the greenhouse and the queen was found dead and partially eaten at the end of April). The known age workers obtained were used for the description of the sting extension response and would not have been recorded for long if provided with RFID tags, given the fate of the colony.

The performance at the colony and individual level could be considered lower-end estimates for the species. In fact, the setup of wasp colonies in the laboratory implied a loss of part of the workforce, change in the original location, colony CO₂ narcosis and a destructive manipulation of the nest structure, arguably having a negative effect on colonies' overall development. Yet, all the above described steps were necessary, and their effects on the individuals might be

negligible. For example, narcosis effects on hymenopterans are mostly unknown, but recent research has showed that CO₂ does not affect *Bombus* foraging activity, while refrigeration (a technique often used also with wasps) does (Poissonnier et al. 2015). Despite being of small dimension, individual tags (numbered tags weight: about 2 mg; RFID tags weight: about 5 mg) potentially constituted an handicap for foraging activities, approximating the weight of a solid or partial liquid load (Archer 1977). The burden of the tags could have had marginal effects, since *V. vulgaris* wasps should be able to carry in flight loads of 30-40 mg (Spradbery 1973; Thomas et al. 1990; Polidori et al. 2013). To estimate to what degree the tags' weight influence activity levels, two tags were glued on a group of workers from the last cohorts of the 2015 colony, but the unfortunate death of the queen compromised the experiment. Further RFID tags miniaturization will be minimizing this type of problem in future research.

The training of *Vespula* workers to the feeding stations resulted challenging, its success being very variable among days (as also noticed with other hymenopterans, including honey bees (von Frisch 1967). Training was majorly constrained by wasps' limited recruiting ability, when compared to honey bees (personal observation, Menzel, personal communication, Fig. 6.1). Specific training methods briefly described in previous literature (e.g. capture of workers at the nest, marking and release at the training station in the desired location (Overmyer and Jeanne 1998)) were replicated with scarce success. In fact, most studies on vespulids' foraging behaviour at the resource location were mostly made on wasp foragers *already* exploiting the resource or arriving from colonies situated in unknown locations (e.g. D'Adamo et al. 2000; Lehrer and Campan 2004; Lozada and D'Adamo 2011). The difficulties encountered in training *V. vulgaris* foragers limited the conclusions when studying foraging activation, not allowing training of numerous workers to different locations. Moreover, the expansion of a data set relative to associative learning capabilities (eavesdropping on ants' trail communication, individual evaluation of food quality in foraging choices) was constrained. One experiment involving training to feeders in the

field, planned and trialed with honey bees, utilizing infra-red thermography, was abandoned given the insufficient time available.

The research project was hindered by technical problems with the RFID machinery and by difficulties encountered in handling the RFID datasets. A substantial amount of time was dedicated to the development and testing of the setup, including nest entrance modules, video recordings, and wasps' rearing techniques. The original project included the study of foragers' behaviour inside the nest and observational nest boxes were designed and built. Given the already high risks present in setting up the experiments (such as the possibility to injure or kill the queen) and considered the additional nest manipulation necessary (separation of nest combs and restructuring of nest on one single level), the nest boxes were not used.



Fig. 6.1 Difficulties in training wasps to feeding stations and difference in recruiting ability between *Vespula* and *Apis*. Honey bees demonstrated their superior resource exploitation efficiency by recruiting in a few hours hundreds of foragers, here limiting resource access to individually marked common wasps (Lincoln, New Zealand, April).

6.4 Further research

Despite the challenges above described, within this thesis I have made some positive advances towards rearing, maintaining and understanding common wasps' behaviour. On the basis of the methodologies and results here presented, further research efforts are encouraged on several fronts.

- I developed rearing and study protocols for individuals and colonies of vespine wasps that could allow important advances in our knowledge of the group and of other hymenopterans. For example, common wasp individuals reared in the laboratory survived for about three weeks, a period of time comparable to the lifespan recorded for the species in the field (Chapters 3, 5). The development of the nest entrance system minimized the workers' traffic jam and RFID record errors (Robinson et al. 2009), allowing high quality video recordings (Chapters 3, 4). Great benefits would come for the development of techniques to start successful colonies in artificial conditions. For example, the comparative study of foundress queens and emerging workers would provide insightful information about the heritability of behavioural traits. Improved training protocols would make the investigation of foraging recruitment simpler.
- Recruitment mechanisms and the possibility of resource location information transfer in social wasp species should be further investigated. Piloting (Chapter 1) could be directly proven tagging entire colonies and utilizing RFID readers at the nest entrance and at the food source. Evidence for pilot flights could be obtained by ruling out alternative explanations for tandem arrivals at the food source. First, specific individuals could be trained to different sites and naïve wasps arrivals observed, to see if they differ by pilot identity and when specific pilots are disturbed (it seems likely that not all pilots would be equally effective). Second, testing how the response to trapping or disturbing a pilot differs from the response to randomly trapping or disturbing any incoming wasp. Ruling out a general

response to disturbance would provide additional, albeit, indirect support for the piloting hypothesis. Signaling among nestmates might take place utilizing particular communication channels and the investigation of foragers' thermal behaviour could provide new insights in this research field. *Vespula vulgaris* is one candidate, but even more promising are other species such as the hornet *Vespa mandarinia* or the swarm founding paper wasps (e.g. *Agelaia* spp.). Groups of workers of these species have been previously observed in coordinated raiding and hunting actions, strongly suggesting nest-based active recruitment (Matsuura & Yamane, 1990; O'Donnell & Hunt, 2013).

- We need to better understand the origins of the extreme intra-colonial variability found both in foraging and aggressive behaviour (Chapters 3, 4, 5). Both intrinsic and extrinsic factors likely account for it. For example, polyandry and different paternity lines might play an important part in explaining the division of labour and the behavioural variability observed among *V. vulgaris* workers. Polyandry is rare among social insects and is hypothesized to have an adaptive significance, determining worker subgroups with different task-related thresholds (Robinson 1992; Strassmann 2001; Goodisman et al. 2007). The importance of individual and social learning in determining behavioural diversification should be also evaluated. Early, even pre-imaginary, experiences might be shaping the behaviour of insects throughout their adult life. Pathogens and parasites could also play an important role in determining division of labour and particular behaviours in wasps, as they do in honey bees (O'Donnell 1997; Kuszewska and Woyciechowski 2014).
- Studies asking whether behavioural variation is an adaptation to sociality are encouraged. It is important to investigate to what extent the variation in behavioural types and the improved foraging performance of specialist workers here observed in *V. vulgaris* translates in measurable fitness ad-

vantages at the colony level. This examination could be achieved via modelling colonies' resource flow with and without worker specialists, or with intra and inter-specific comparative studies within the genus *Vespula*. In fact, closely related species within this peculiar group can show colonies of very different size (two orders of magnitude) and new evidence was provided for marked interspecific differences and strong individuality in behaviour (Parrish and Fowler 1983; Kim et al. 2007; Wilson-Rankin 2014).

- The lifelong behavioural differences found among colony members could be consistent across context and there might be a link between different behavioural traits (Chapters 3, 4, 5). For example, more active, bolder foragers could be also more aggressive and more inclined to nest defence. Testing this hypothesis would allow speculations on the existence of different personalities and the assessment of their biological significance in a wider perspective (Wolf et al. 2007; Pinter-Wollman 2012b). Further studies could evaluate the importance of personality in the evolution of division of labour within these highly social insect species (Grinsted and Bacon 2014).
- An unexplored hypothesis on social wasps' temporal polyethism awaits to be tested: that the polyethic transitions observed in the common wasps (Chapter 3), strikingly similar among *Vespula* colonies and species (Potter 1964; Hurd et al. 2007; Kim et al. 2012), could be explained by the relatively short span of the colonial stage and by the changing resources' needs during their rapid colony development (Jeanne 1991).
- I encourage the study of the cognitive and learning capabilities of wasps, utilizing the classical conditioning the sting extension response (SER) described and scored in the present work (Chapter 5). Aversive learning through SER conditioning was demonstrated with both olfactory and visual association in honey bees (Vergoz et al. 2007; Mota et al. 2011). Intriguing hypothesis could be tested, including (i) Differential species-specific

learning capabilities, for example associated to different ecology or social complexity (ii) the possibility of inter-specific eavesdropping in foraging communication, and (ii) intra-specific differences in the learning capabilities between populations in the native and invaded range.

6.5 Conclusions

Overall, my research emphasizes the role of individuality in animal societies and their complexity. Indeed, the striking variability found between wasp workers prevails over the description of any average phenotype within a colony (Chapters 3, 4). Inter-individual differences in common wasps' foraging and stinging behaviour were only partially explained by wasps' age and size (Chapters 3, 4, 5). The association between individual task specialism and efficiency suggest that division of labour within societies has adaptive benefits (Chapter 4). Social information played a role in the co-ordination of foraging activity at the colony level (Chapter 2). Direct and indirect experience and learning processes appeared to shape wasps' behaviour throughout their life (Chapters 2, 3, 4, 5). Further research is encouraged to develop social wasps' rearing and training techniques and better understand recruitment systems within this group of social insects, their division of labour and cognitive and learning capabilities.

The findings here summarized contribute in our understanding of the ecology and evolution of individual specialization and division of labour within social insect colonies, focusing on their long neglected variability. Additionally, my study makes a significant contribution by broadening our knowledge of the largely unknown wasp societies, posing the basis for exciting further research.

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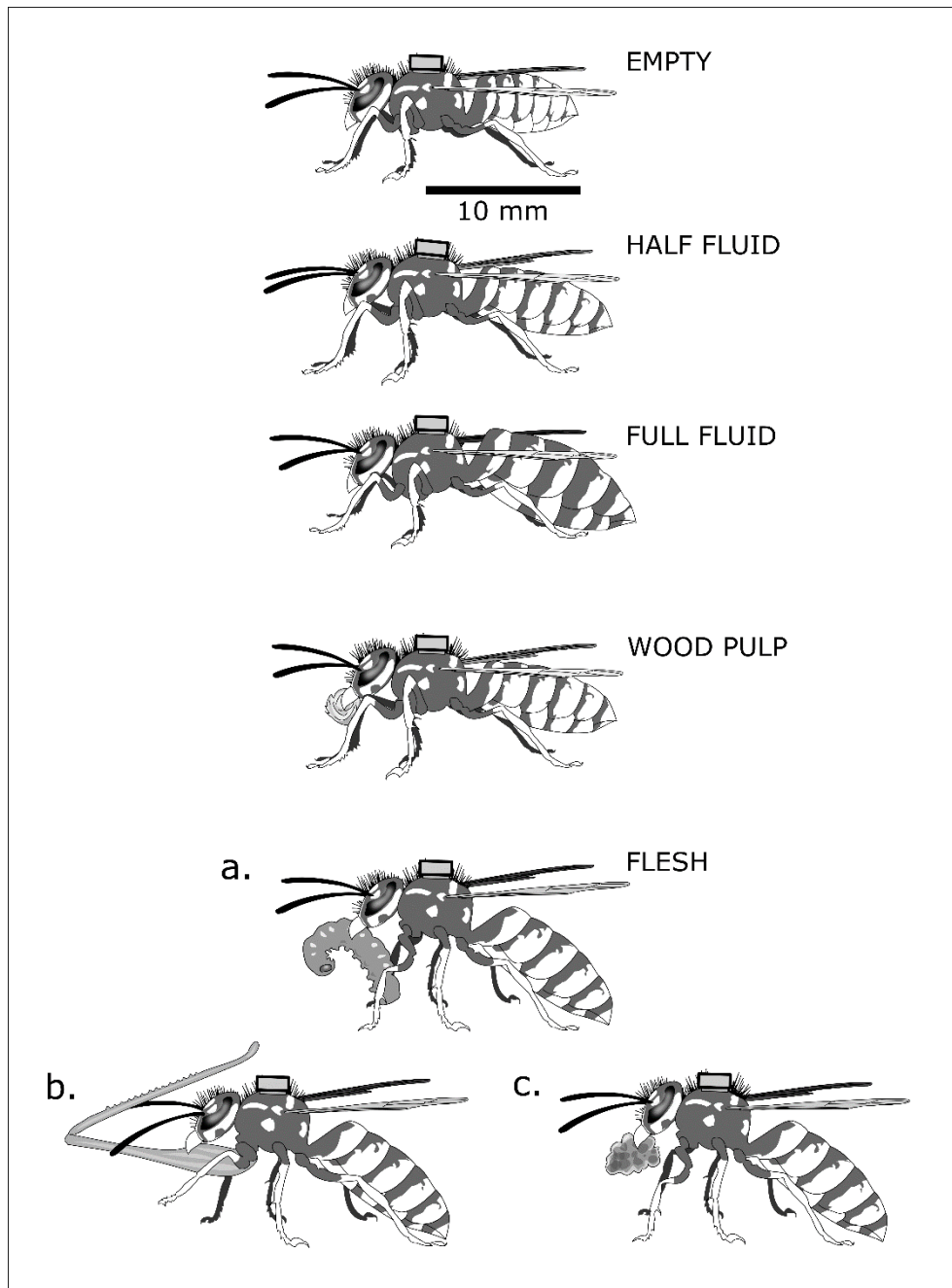


Plate I Common wasp (*Vespula vulgaris*) female, adult workers. Observable behavioural categories for wasps returning to the nest after a foraging trip: empty; with fluid, partially loaded; with fluid, fully loaded; with a wooden pulp load; with a flesh load (a. entire insect prey b. insect body part c. indistinguishable, malaxated flesh, coming from hunting or scavenging activities). Individuals are represented with an RFID tag glued on the thorax. Drawings by David Young.