VNiVERSiDAD
Đ SALAMANCA

FACULTAD DE BIOLOGÍA
Departamento de Biología
Animal, Parasitología, Ecología,
Edafología y Química Agrícola
(Área de Zoología)

Foraging, nest establishment and chemistry of parasitism in digger wasps (Hymenoptera: Crabronidae: *Bembix*, *Stizus*)

PhD Dissertation Summary

European Doctorate

Yolanda Ballesteros Álvarez

Supervisors: Dr. Josep Daniel Asís Pardo, Dr. Josep Tormos Ferrando, Dr. Laura Baños Picón and Dr. Carlo Polidori.

Salamanca, 2013

CONTENTS

INTRODUCTORY CHAPTER

---

1. STUDY SUBJECT AND TOPICS

1.1. The sand wasps: general aspects of their biology

Bembicinae (Hymenoptera: Crabronidae), commonly known as “sand wasps”, is a subfamily of about 1,700 species (Pulawski, 2012). The subfamily is worldwide distributed and particularly diverse in the desert areas of southwestern United States, southern Africa and Australia (Evans and O’Neill, 2007). They are solitary wasps, with females provisioning their nests with paralyzed prey to feed their larvae (with the exception of *Microbembex* Patton, 1979, whose females bring dead or disabled prey to their nests, Evans and O’Neill, 2007), yet those of a few genera (e.g. the Nyssonini *Nysson* Latreille, 1802, *Zanysson* Rohwer, 1921 and *Acanthostethus* Smith, 1869, as well as the Stizini *Stizoides* Guérin-Méneville, 1844) feed (as cleptoparasites) at the expense of other wasps (Evans, 1966a). Females dig nests exclusively in the ground (O’Neill, 2001), using their mandibles and forelegs. These nests are composed of a burrow, at the end of which one to various brood cells (Fig. 1-a) can be found. In each cell, the female deposits an egg, together with the necessary number of prey to complete the development of the larva (Evans and O’Neill, 2007).

Nests are established in sandy soils of variable compactness, and appear frequently gathered in dense aggregations (Evans and O’Neill, 2007). In occasions, structures called *accessory burrows* (Figs. 1-a, 1-b), i.e. short galleries that presumably confound nest-searching parasites (Evans, 1966b; O’Neill, 2001), are built in close vicinity of the true nests. Accessory burrows have been occasionally reported from

Stizus Latreille, 1802 and frequently observed in *Bembix* Fabricius, 1775 (Evans and O'Neill, 2007).

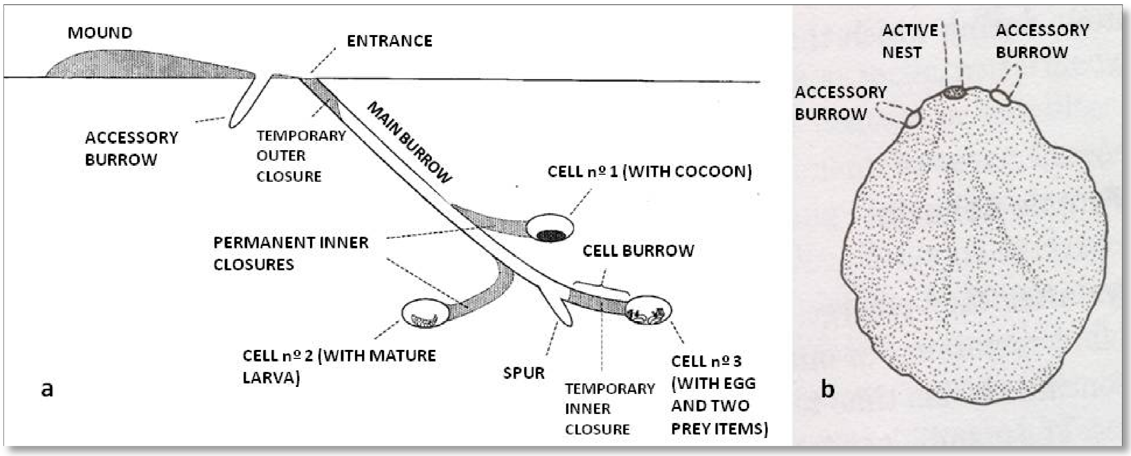

Figure 1. **a**. Idealized representation of the nest of a Bembicinae in lateral view. The number of cells may vary depending on the species; similarly, outer and inner closures, mounds and accessory burrows may be present or absent. **b**. Idealized mound of a Bembicinae nest in upper view, with two possible accessory burrows in lateral position, and the entrance to the active nest in central position. Figures taken from Evans and O'Neill (2007) and Evans and O'Neill (1988), respectively.

Although in some species the sand mounds originated during nest digging are dispersed by the female (Evans, 1957; Evans and O'Neill, 2007), in the majority of the Bembicinae species the mounds are not dispersed, in such a way that they are conspicuous (Evans, 1966a) and visible at the nest entrance (Figs. 1-a, 1-b).

Prey are paralyzed by venom administration through the sting and, in the Stizini and Bembicini, transported in flight to the nest, in such a way that provisioning, as is usual in solitary wasps, represents an energy-demanding activity (Field, 1989; Strohm and Linsenmair, 1999; Strohm and Marliani, 2002). The provisioning patterns observed in sand wasps fit in the strategy of the *central-place foragers*, which travel from a central place (their nest) to different places to get provisions, going back to their nest with a single prey per journey (Stephens and Krebs, 1986). Many species maintain temporary inner and/or outer closures when the female is away or when she prepares to spend the night inside the nest (Evans and O'Neill, 2007).

Solitary wasps can contribute prey to their nests following two basic models, yet a wide variety of intermediate strategies has been described between them. On one hand, in the *progressive* model, the provisioning activity substantially overlaps in time with the larval stage of the brood, and the female closes the nest just before the larva begins to spin its cocoon (Evans, 1966a); this way, the female is able to perform a monitoring of her larva and to carry a specific amount of food to the nest, depending on its daily requirements. In the *massive* model, on the other hand, all the prey are contributed to the nest before the egg hatches, in such a way that there is no contact between the female and her larva (Evans and O'Neill, 2007).

When the cell provisioning has been completed, the female closes it (filling the access to the main burrow with sand, placing a permanent inner closure, Fig. 1-a), and when all the cells have been provisioned, she also closes the main nest burrow permanently. The larva, on its part, will begin to spin its cocoon when it has finished eating the whole provision, next reaching the pre-pupal stage (Evans and O'Neill, 2007). Only a nest is provisioned at the same time by a given female.

Male behaviour is in general less complex than that of the females. They patrol in search of emerging virgin females ("scramble competition", Evans and O'Neill (2007)) or defend small territories within emergence areas. Females copulate only once in their lifetime and are receptive immediately after their emergence. Sand wasps are proterandrous, male activity peaking during the period of emergence of the females; then, the abundance of males decreases in 2 to 3 weeks, in such a way that they may be absent in more advanced stages of the flying season (Evans, 1966a). Males of *Bembix* generally compete for females through scramble competition (but see Dodson and

Yeates, 1989 and Larsson and Larsson, 1989), while in some *Stizus* territoriality has been reported (Asís *et al.*, 1988; 2006). In *Stizus continuus*, as well as in some species belonging to the genus *Bembix*, competition for females may be so extreme that males dig in points where they detect females that are about to emerge, probably driven by chemical and/or vibrational cues (Schöne and Tengö, 1981; Larsen *et al.*, 1986), in occasions forming a characteristic “mating ball”, composed of numerous males clumping around a recently emerged female (Evans, 1957; Asís *et al.*, 1988).

1.1.1. The genus *Bembix*

Bembix (Fig. 2) is the largest of the genera of Bembicini, including *ca*. 350 species (Pulawski, 2012). It is a widespread genus, with species distributed in all zoogeographic regions, 50 of which are present in the Palaearctic Region (Bohart and Menke, 1976).

Females of all European *Bembix* species capture prey from the order Diptera to feed their larvae (Evans and O’Neill, 2007; Ballesteros *et al.*, 2012), yet other groups of insects, such as Lepidoptera, Odonata, Neuroptera and Hymenoptera have been cited as prey of the African or Australian species (Evans, 1957; Evans and Matthews, 1973). After the nest closure, the larva completes its development within one or two days (Evans, 1957), and then it begins to spin its cocoon.

Within the genus, species with both multivoltine and univoltine life cycles can be found. In multivoltine species, the larva spends just one month inside its cocoon (about half a month in the pre-pupal stage and the rest of the time as a pupa); nevertheless, in univoltine species (as well as in the last annual generation of multivoltine species), the larva remains in the pre-pupal stage until the following spring, when it pupates and emerges, probably as a response to temperature changes (Evans, 1957).

Bembix merceti Parker, 1929 is endemic to the Iberian Peninsula, where the females establish their nests in sparse aggregations located in sandy soils. Nests are unicellular, with the cell at 7.5–11 cm depth; provisioning is progressive, and inner and outer closures are present. It takes a female 5-6 days to complete a nest (Asís *et al.*, 1992, 2004). Different species from the families Sarcophagidae, Tachinidae, Muscidae, Bombyliidae, Stratiomyidae, Calliphoridae, Syrphidae and Tabanidae have been cited as prey (Asís *et al.*, 1992, 2004; Ballesteros *et al*., 2012).

Bembix zonata Klug, 1835 is distributed in the south of Europe. Females dig unicellular nests in sandy soils, with cells at 8–11.5 cm depth, and perform a progressive provisioning (Asís *et al*., 2004). For this species, prey from the families Syrphidae, Anthomyiidae, Bombyliidae, Calliphoridae, Muscidae, Tabanidae, Asilidae and Therevidae (Bernard, 1934, 1935; Asís *et al*., 2004; Ballesteros *et al*., 2012) have been reported.

The females of *Bembix sinuata* Panzer, 1804, a species distributed in southern Europe and northwestern Africa, build unicellular nests in sandy soils, with cells at 7–22 cm depth, maintaining inner and outer closures and progressively provisioning their brood cells. This species has been observed to contribute flies from the families Calliphoridae, Muscidae, Syrphidae and Tachinidae (Asís *et al.*, 1992).

1.1.2. The genus *Stizus*

The genus *Stizus* includes more than 100 species (Pulawski, 2012), and is widely distributed through temperate and tropical regions, yet it has not been cited from South America, Australia and South East Asia (Evans and O'Neill, 2007).

Stizus continuus (Fig. 3) has a palaearctic distribution, being bivoltine in southern Europe (Asís *et al*., 2006; Polidori *et al*., 2008, 2010a). Females dig pluricellular nests (with 3–8 cells, at 8–18 cm depth). The existence of accessory burrows has been documented (Asís *et al*., 1988). The females establish their nests in salt marshes, forming large aggregations, and capture nymphs or imagoes from different orthopteran families, such as Acrididae, Pyrgomorphidae or Tettigoniidae (Asís *et al*., 1988, Polidori *et al*., 2009).

Figure 2. Marked *Bembix sinuata* female at the nest entrance, with a prey (Diptera) below her body, held with her mid legs. Picture by J. D. Asís.

Figure 3. Marked *Stizus continuus* female at the nest entrance, with a prey (Orthoptera) held with the mid legs of the wasp. Picture by D. Santoro.

Even though the provisioning is massive in the vast of the species of the genus, Asís *et al*. (1988) observed that some *S. continuus* females brought prey to cells where the egg had already hatched, practicing a special kind of massive provisioning, called "slow massive provisioning" (Genise, 1982). Females place temporary closures during their absence, or when they prepare to spend the night inside the nest.

1.2. Natural enemies

In general, solitary wasps are threatened by different kinds of natural enemies (O'Neill, 2001), that can operate through diverse strategies.

The females of cleptoparasites lay their eggs or larvae within alien nests, so that their brood eats the provisions stored by the host. This one is the strategy employed by different hymenopteran species belonging to the families Chrysididae, Pompilidae and Sapygidae, or to the Spheciformes group, as well as by some dipterans belonging to the family Sarcophagidae (O'Neill, 2001).

The larvae of parasitoids, on the other hand, develop at the expense of the larva or the pre-pupa of the host (Godfray, 1994), a strategy used by the hymenopterans of the family Mutillidae and by some species of Chrysididae, and also by the dipterans of the family Bombyliidae.

There is also a number of prey thieves, that subtract the provisions stored in alien nests and transport them to other places to utilize them as their own provisions. In this category, conspecific females and other species of solitary wasps, as well as ants, can be included (Evans and O'Neill, 2007).

Of special relevance in the present investigation are the natural enemies belonging to the family Chrysididae (Hymenoptera), and also to a group of Diptera included in the family Sarcophagidae.

Around 3,000 chrysidid (Hymenoptera: Chrysididae) species have been described (Kimsey and Bohart, 1990), of which 170 are present in the Iberian Peninsula (Mingo, 1994). Chrysidids possess metallic colours and a strongly sclerotized cuticle, most of them being able to roll in a protective position when threatened. They are commonly known as “cuckoo wasps”, due to the strategy they use to rear their brood: adult females, after having been inseminated, look for a nest of a suitable host, keep watching the host female nesting activity, and gain access to the nest to lay an egg when the female is away (Enslin, 1929; Clausen, 1940; Danks, 1970; Polidori *et al.*, 2010b).

The subfamily Chrysidinae, to which the genus *Parnopes* Latreille, 1796 (studied here) belongs, attacks aculeate hymenopterans (Gauld and Bolton, 1988), and includes both species that act as cleptoparasites and species that behave as parasitoids. In the case of the parasitoid species, two oviposition strategies can be found, depending on the developmental stage of the host: oviposition can be carried out during or after the cell provisioning process, in such a way that the egg of the chrysidid hatches and the larva remains on its first instar until the host larva completely develops; or can be otherwise performed when the host is on the pre–pupal stage. Although *Parnopes* behaves as a parasitoid, the kind of oviposition strategy employed by it is still not known.

Among digger wasps, the parasitism of the pre-imaginal stages by Chrysididae is not uncommon (Evans and O’Neill, 2007), some Chrysidinae species having been cited as natural enemies of *Bembix* (Asís *et al.*, 1992; Tormos *et al.*, 2009). *Parnopes grandior* (Pallas, 1771), the only species of the genus in the Iberian Peninsula (Mingo, 1994), has been reported as a brood parasite of *Bembix sinuata* and *B. zonata* (Asís *et al.*, 1992), as well as of several other *Bembix* (Abeille, 1878; Grandi, 1930, 1934; Semenov and Nikolskaya, 1954).

Satellite flies (Diptera: Sarcophagidae: Miltogramminae), on their part, can act as cleptoparasites of Spheciformes wasps (Linsley and MacSwain, 1956; Ristich, 1956; Evans, 1966a; Evans and Hook, 1986). Adult females of satellite flies constantly patrol the nesting areas of their hosts, looking for nest entrances, or waiting on perches for prey-carrying host females, larvipositing within the nest on the host or on the provisions that are being transported to the nest (Newcomer, 1930; Ristich, 1957; Evans and O'Neill, 1988; Spofford and Kurczewski, 1990; Alcock, 2001).

The larvae of satellite flies quickly consume the paedotrophic material stored in the brood cell for the host, in such a way that the host larva usually starves (O'Neill, 2001). Nevertheless, in some *Bembix* species it has been observed that both the host and the fly larvae were present in the cells (Asís *et al*., 1992). This could be due to the kind of provisioning (progressive) performed by *Bembix,* that offers the possibility of tuning the amount of food contributed to the nest on the basis of the daily requirements of the larva, this way compensating for the higher prey consumption rate in the case of miltogrammine-parasitized cells.

1.3. The sand wasps as models in behavioural ecology

Solitary wasps have been one of the preferred study objects of naturalists and ethologists for more than a century. Starting with Fabre and his *Souvenirs Entomologiques* (1879), where the author studied aspects such as the homing or the innate behaviours of solitary wasps; and with Ferton, who largely contributed to the knowledge of the biology of the Spheciformes in his numerous publications (1894, 1895, 1899, 1901, 1902, 1905, 1908, 1909, 1910, 1911, 1912, 1914, 1920), a great number of investigations have demonstrated that solitary wasps are suitable subjects for the development of behavioural studies in the field. For example, classical ethologists utilized various Spheciformes genera, such as *Philanthus* Fabricius, 1790 (Tinbergen, 1932, 1935), *Ammophila* Kirby, 1798 (Baerends, 1941) or *Bembix* (van Iersal, 1952) as ethological models to carry out studies related to the homing and hunting behaviour, to the nesting behaviour, and to the homing, respectively. More recently, Grandi (1961) provided data on the nesting behaviour of various fossorial species, and Evans (1957, 1959, 1966a) and Alcock (1975a, b, c) have addressed behavioural ecology studies, using Spheciformes wasps as models.

Thus, traditionally, behavioural ecologists and evolutionary biologists have considered solitary wasps as good models to study the evolution of foraging and parental strategies, as well as other topics related to mating systems, thermoregulation and eusociality (O'Neill, 2001).

In any organism (sand wasps included), energy acquisition is a basic requirement for the survival and the proper working of vital processes. For this reason, the identification of the factors shaping the trophic niche has taken up, from decades ago, a paramount place within the studies of the ecology of animal species (Stephens and Krebs, 1986; Werner and Sherry, 1987; Smith, 1990; Araújo and Gonzaga, 2007; Araújo *et al*., 2010). Ecologists agree that the diet of an organism is a fundamental aspect of its ecological niche, whose study and quantification are essential to know the basic ecology of a species (Sih and Christensen, 2001). If descriptive studies (at a species- or population- level) on the prey types captured by different sand wasp species (Evans and O'Neill, 2007) are numerous, this group has, additionally, a huge potential to carry out more precise studies in relation to prey capture, from newer and more realistic perspectives. In particular, these wasps possess traits that make them suitable for the study of resource partitioning at an intra-specific or intra-populational level, as well as for the assessment (through the *ODT*) of the potential performance of an optimal predation (see below). Their suitability for these kind of studies lays, on one hand, on their behaviour as *central-place foragers*, that allows the assignment of different kind of prey to specific females, as well as the obtaining of various prey items per nest (Polidori *et al*., 2005, 2010c; Santoro *et al*., 2011), increasing the reliability of the observations; on the other hand, body size variation is usually large enough within a given population of wasps as to detect fitness differences potentially related to the size of the wasps (included the provisioning-related fitness differences) (O'Neill, 2001; Evans and O'Neill, 2007; Coelho, 2011).

If food acquisition is fundamental for the survival of organisms, the relationships between hosts and natural enemies are among the most important biotic relationships (Strohm *et al*., 2008). In particular, the nests of brood-caring wasps and bees (as is the case of *Bembix* and *Stizus*) are interesting targets to parasites (Wilson, 1971; Jacob-Remacle, 1986; Evans and O'Neill, 1988), owing to the great amount of nutritious resources stored there. The interests of natural enemies and hosts are largely divergent, as the first ones must develop strategies to evade the detection by the hosts, while the second ones must improve their mechanisms to protect their brood, including a prompt recognition of natural enemies (Tengö and Bergstrom, 1977; Rosenheim, 1988; Quicke, 1997; Strohm *et al*., 2001, 2008). Thus, an "arms race" between both is originated (Dawkins and Krebs, 1979; Davies *et al*., 1989; Foitzik *et al*., 2003; Lyon, 2004; Brandt *et al*., 2005). As insects heavily rely on chemical cues to get information of their surrounding environment (Godfray, 1994; Jackson and Morgan, 1993; Herzner *et al*., 2005), it seems reasonable that these "arm races" have, at least, a component of chemical nature, cuticular hydrocarbons (CHCs) being particularly important for the development of recognition functions (Blomquist and Bagnères, 2010). The CHCs, non-polar lipids that fully cover the insects' cuticle, develop the main function of avoiding desiccation (Lockey, 1988); nevertheless, they have the important secondary function of acting as semiochemicals for the recognition among two or more individuals (Howard and Blomquist, 2005; Blomquist, 2010). The study of the CHCs has proven useful in explaining the parasitism relationships of a variety of insects (Bagnères and Lorenzi, 2010), and is used here to investigate the parasitism of a cuckoo wasp towards some species of sand wasps.

Certain kinds of parasitism have been proposed as factors that, under specific circumstances (Wcislo, 1986; Evans and O'Neill, 1988), could determine the trend of numerous sand wasps to nest in aggregations (Evans and O'Neill, 2007), yet they are not the only factors that may cause such aggregation. Other causes, such as those related to the *selfish herd* hypothesis (Wcislo, 1984; Larsson, 1986) and the shortage of suitable nesting patches (Brockmann, 1979), as well as philopatry processes (Yanega, 1990) and female-female attraction (Polidori *et al*., 2008), have been suggested to be involved in the establishment of nests in aggregations.

1.4. The *Optimal Diet Theory* (*ODT*)

The journey to the foraging place, as well as the search and capture of prey items, are expensive in terms of energy consumed and, as has been highlighted before, the expense is particularly elevated in solitary wasps. The *ODT*, first proposed by Emlen (1966) and MacArthur and Pianka (1966), is a good starting point to assess if an individual will select its prey trying to optimize its energy investment, under the hypothesis that predators forage in an optimal way.

The *ODT* has been used to assess the type of diet selected by a wide variety of animals, both vertebrates and invertebrates, and in consumers of animal, as well as vegetal, matter (Sih and Christensen, 2001). According to the *ODT*, in *central-place foragers*, as is the case of sand wasps, a minimum acceptable prey rule exists: try to capture a prey and go back to the central place if the prey value compensates for the trip and search energy investment. Provided that prey are distributed in patches in the environment, and also that these patches can be located at different distances from the central place, the energy costs involved in the capture of a prey item would depend, among other factors, on the distance from the patch to the central place. For this reason, the energetic value threshold that makes a prey acceptable will increase as the distance from the central place to the foraging patch grows, in such a way that it would be expected that predators capture prey items of a higher energetic value in further patches (Pyke, 1984). In the same way, according to the *ODT*, larger individuals, able to transport larger weights (Marden, 1987), will optimize their captures by predating upon bigger prey (of a higher energetic value), as long as the weight of the prey doesn't dramatically affect the costs involved in their capture.

It is necessary to take into account the influence that the biometric traits of the body parts involved in flight activity (wing area, flight muscle mass or body mass) may exert on the successful transportation of a bigger or smaller amount of weight (Norberg and Rayner, 1987; Chai and Srygley, 1990; Domenici, 2001; Almbro and Kullberg, 2008, 2009; Vogel, 2008). Following Marden (1987), the two main parameters determining the flight capacity are the ratio of flight muscle mass to total body mass (*Flight Muscle Ratio*, *FMR*), and the ratio of body mass to total wing area (*Wing Loading*, *WL*), in such a way that the occurrence of prey optimization could be shaped by the limitations that these two parameters impose on the wasps, as well as by the difference among the *FMR* or *WL* values of predators and prey items (Chai and Srygley, 1990; Hedenström and Rosén, 2001).

However, currently there is not an agreement about whether solitary wasps do, or do not, optimize their provisioning flights to try to compensate for the energy invested on them. For example, in the crabronids *Sphecius speciosus* (Drury, 1773), *Tachytes chrysopyga* (Spinola, 1842) and *Bembix troglodytes* Handlirsch, 1893, the existence of optimization has been ruled out (Hastings, 1986; Coelho, 1997; Grant, 2006; Coelho *et al.*, 2008), while in the sphecids *Palmodes laeviventris* (Cresson, 1865) and *Sphex ichneumoneus* (Linnaeus, 1758), as well as in the crabronid *Clypeadon laticinctus* (Cresson, 1865) either direct correlations between wasp and prey mass or the selection of the biggest prey among those available have been observed, which suggests that in those wasps the presence of optimization cannot be completely discarded (Gwynne and Dodson, 1983; Alexander, 1985; Coelho and Ladage, 1999); moreover, the crabronid *Sphecius convallis* Patton, 1879 has been cited as the most ideal flying predatory wasp (Coelho, 2011), preying in an optimal way.

Thus, it seems necessary to obtain more data to interpret properly the foraging patterns observed in the group of the solitary wasps, in such a way that, in the present study, a new investigation is performed within the framework of the *ODT*, utilizing wasps of the genus *Bembix*, to try to improve the current understanding of the aspects related to the optimization of the foraging behaviour.

1.5. The concept of Individual Specialization

For decades, species, or populations of a species, have been considered to be composed of ecologically equivalent individuals, which made use of the same set of resources (Bolnick *et al.*, 2002). Thus, in the past, niche has been treated as a property of the species, or populations, as a whole (Colwell and Futuyma, 1971; Pielou, 1972). Nevertheless, there is nowadays a strong tendency towards the consideration of the existence of individual specialization in the studies of trophic ecology, as there is evidence that it is widely extended in the animal world (both Bolnick, 2003 and Araújo *et al.*, 2011 have published reviews on the occurrence of this phenomenon in animals). It can be considered that an individual behaves as a specialist when it consumes a portion of resource types that is significantly smaller than the portion consumed by its population as a whole (Bolnick, 2003); or when its niche shows little overlap with that of its population (Bolnick *et al*., 2002; Svanbäck and Persson, 2004). Thus, populations or species that are seemingly generalists, could be actually constituted by individual specialists that consume smaller subsets from the total population niche (Bolnick *et al*., 2007). Additionally, individual specialization may have important effects on the dynamics and stability of populations, and on the species coexistence (Lomnicki, 1988; Kendall and Fox, 2002; Lichstein *et al*., 2007; Okuyama, 2008; Lankau, 2009).

Given that the study of individual specialization constitutes a relatively new approach, the number of wasps for which it has been quantified is still low (Araújo and Gonzaga, 2007; Polidori *et al*., 2010c, 2011; Santoro *et al*., 2011), and, in any case, the reasons causing the partition of resources among the individuals of a population or species are still largely unknown (Araújo *et al*., 2010). One of the aims of the present study is to provide information on the factors involved in resource partitioning at an intra-populational level. Additionally, the data have been analysed from new perspectives, such as those based on network theories (Araújo *et al*., 2008; Pires *et al*., 2011) or on the correlation of the morphological distance with the diet dissimilarity (Bolnick and Paull, 2009), which have proven useful in similar investigations carried out in other taxa, particularly vertebrates, and that have been only recently applied to ecological studies on predatory insects (Polidori *et al*. 2013).

1.6. Nesting in aggregations and spatial patterns

In fossorial hymenopterans, the aggregation of nests in more or less restricted areas is not dependent on the existence of a social condition, appearing in solitary and eusocial species, as well as in species where the females share their nests (Evans, 1955;

Evans and O’Neill, 1988; McCorquodale, 1989; Potts and Willmer, 1997, 1998; Casiraghi *et al*., 2003; Polidori *et al.*, 2006a).

Many species of sand wasps show a trend towards the establishment of their nests in aggregations (Evans and O’Neill, 2007). The evolutionary reasons proposed to explain such tendency are various, and include both biotic and abiotic components: for example, aggregation could be originated owing to the fact that the area suitable for nesting has a limited extension, as well as to the clustered distribution of key resources (Michener *et al*, 1958; Brockmann, 1979; McCorquodale, 1989), that can lead to philopatry (Evans and Hook 1986; Yanega 1990, Polidori *et al.*, 2006b).

Both costs and benefits can be found associated to the aggregation of the nesting females. On one hand, among the costs, the potential interference among neighbouring females while digging or provisioning their nests (Lüps, 1973), the accidental provisioning of alien nests (Evans and Mattews, 1973), a higher risk of prey cleptoparasitism by females (Field, 1992; Casiraghi *et al*., 2003), a higher competition for limited resources (Asís *et al*., 2006; Polidori *et al*., 2006b) and an increased pathogen transmission (Brown and Brown, 1986) have been highlighted. The advantages, on the other hand, would include the opportunity to benefit from the potential strategy against parasitism or predation that nesting in aggregations may represent (the *selfish herd* hypothesis, or the *many eyes* hypothesis, Hamilton, 1971; Treherne and Foster, 1980; Evans and O’Neill, 1988; Spofford and Kurczewski, 1992), or a higher probability of mating (Nordell and Valone, 1988).

Other factors, such as the previous establishment of individuals within a specific area, may favour the attraction of conspecifics towards this area, yet the presence of those individuals could be interpreted as the existence of low predation risk (Rudolf and

Rödel, 2005). Additionally, the presence of certain natural enemies could be related to the host density is some occasions (Rosenheim, 1990; Polidori *et al*., 2010b).

The kind of spatial pattern exhibited by the nests within the aggregations can be variable. Thus, nests can be spatially organized in three different patterns: random, regular or in clusters. Although the factors favouring one or another kind of distribution pattern have not been studied in depth, it is known, for example, that intra-specific competition is a factor determining the distribution of nests in a regular way (Brockmann, 1979; Rubink, 1982). Moreover, the scale at which observation is being carried out is of major importance to achieve a correct diagnosis of the kind of pattern exhibited (Potts and Willmer, 1998; Polidori *et al*., 2008). Excluding the widely studied ants, in-depth statistical approach to the study of the spatial distribution of nests has been poorly developed in fossorial hymenopterans, being limited to four species of bees and three species of solitary wasps (Rubink, 1982; Kukuk and Decelles, 1986; Riddick, 1992; Potts and Willmer, 1998; Boesi *et al*., 2007, 2009; Polidori *et al*., 2008). Therefore, it seems necessary to provide new data that help to clarify the kinds of spatial patterns followed by fossorial hymenopterans when establishing their nests, as well as to unveil the factors leading to the adoption of such patterns.

1.7. The CHCs, cuticular compounds of a significant importance

The cuticular hydrocarbons, or CHCs, coat the whole external surface of insects, forming a continuous layer, and their main function is to avoid or to reduce desiccation, abrasion or infections (Hadley, 1981; Lockey, 1988; Buckner, 1993; St. Leger, 1995; Gibbs, 1998; Martin *et al*., 2010). Nevertheless, they develop an important secondary function: they act as semiochemicals for the recognition among individuals (Howard and Blomquist, 2005; Blomquist, 2010) at a species, sex, caste or reproductive stage level, nest mate discrimination, or recognition of parasites and parasitoids (e.g.

Greenberg, 1979; Howard, 1982; Takahashi and Gassa, 1995; Singer, 1998; Lahav *et al.*, 1999; Ruther *et al.*, 2002; Gamboa, 2004; Howard and Blomquist, 2005; Lucas *et al.*, 2005). Following Blomquist (2010), the alkanes (whose simplest organizations include chains composed of carbon and hydrogen) and the alkenes (of chains that exhibit one or more double bounds) are two of the three great classes of cuticular hydrocarbons.

The females of the brood parasites of hymenopterans frequently must enter the nests of their hosts in order to deposit their eggs or larvae, in such a way that traces of those females may remain into the nests. As a response to such traces, the host female could abandon her nest or destroy the alien eggs (Rosenheim, 1988; Kimsey and Bohart, 1990), or inflict damage on the parasitic female. As insects frequently employ chemical cues (often times, CHCs) in their recognition and location processes (Jackson and Morgan, 1993; Godfray, 1994; Herzner *et al.*, 2005), it is paramount for a natural enemy to remain chemically unnoticed inside the nest of its host.

Even though the parasite-host relationships are among the most important biotic relationships (Strohm *et al.*, 2008), they have been poorly studied at a chemical level in the case of the brood parasites of brood-caring hymenopterans (Hefetz *et al.*, 1982; Strohm *et al.*, 2008). Thus, it is necessary to know the chemical relationships of a greater number of host-parasite systems in this kind of hymenopterans, a need that we aim to satisfy in the present study.

2. GENERAL METHODOLOGY

2.1. Study area

The field work for the development of the present study was carried out at two localities: mainly in Almarail (Soria, Spain) (Fig. 4), and to a lower extent in the Dehesa del Saler Natural Park (Valencia, Spain) (Fig. 5), during 2007, 2008, 2009, 2010 and 2011. All the data were collected during the summer period, as it is in this season when the wasps are active in the nesting areas, in the adult stage.

All the data concerning the studied *Bembix* species were collected within a plot in Almarail (41°17’N; 4°06’O) (Figs. 4 and 6), a locality placed 28 km south of Soria (central Spain), during the years 2008 to 2011. The plot is a sandy area devoid of thick vegetation, with some sparse specimens of plant species belonging to the families Resedaceae, Lamiaceae, Asteraceae and Scrophulariaceae, and contiguous to a *Pinus pinaster* Aiton, 1798 (Pinaceae) pine-tree forest, in the vicinity of the Duero river (which flows at a distance of about 100-200 m). In the surrounding environment, there is a predominance of cereal crops, being present some abandoned plots, which constituted the study area.

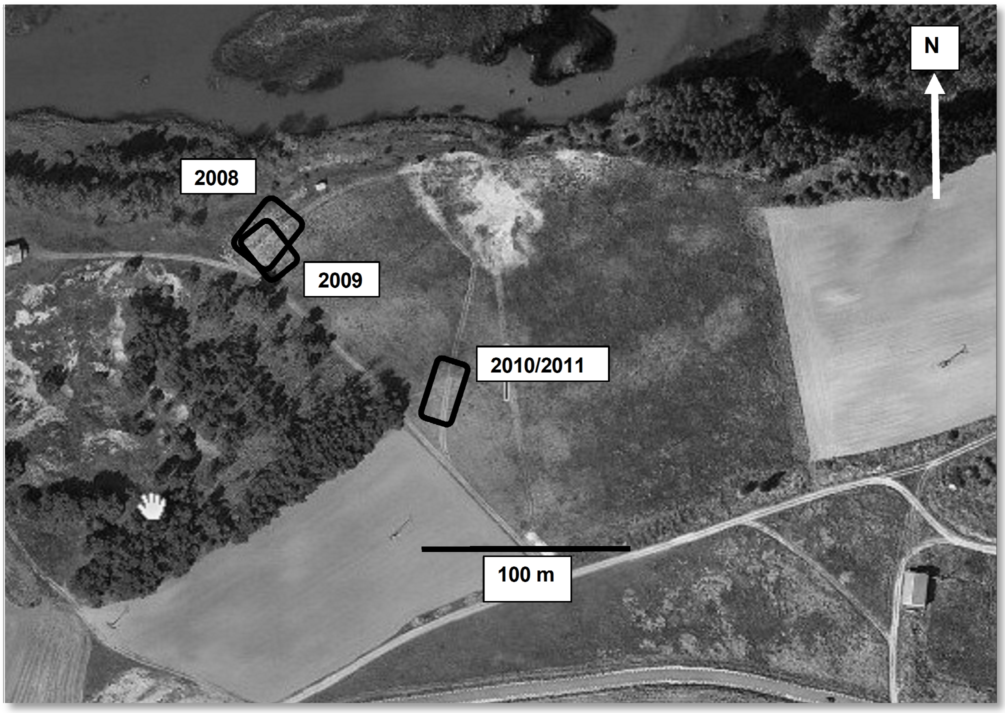

Figure 4. Aerial view of the plot where the different study areas were established in Almarail (Soria, Spain), between 2008 and 2011. Note that in the last two years, the nesting area was displaced *ca.* 100 m to the south-east with respect to the previous years.

Data collection of *Stizus continuus* was performed at “La Mallada Larga” (39°21’N; 0°18’O) (Figs. 5 and 7), within the Natural Park of Dehesa del Saler, a salt marsh situated 13 km south of Valencia (south-eastern Spain), during the summer of 2007. The area is characterized by the presence of wet sandy soil, which is subject to intermittent periods of floods during the winter and desiccation during the summer, originating a superficial salt crust in that season. The study area presents low sized vegetation, with scattered specimens of plants belonging to the families Amaranthaceae, Poaceae and Juncaceae. Nevertheless, some small patches show bare soil, where aggregations of *Stizus continuus* and, in much lower degree, of other sand wasps, are located.

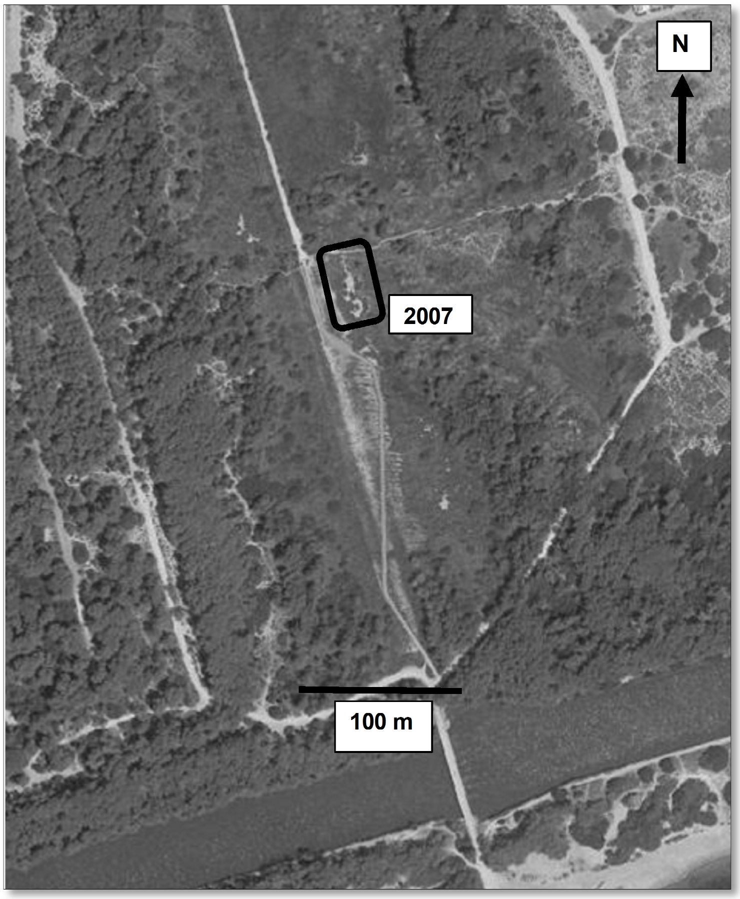

Figure 5. Aerial view of the plot where the study area of “La Mallada Larga” (Valencia, Spain) was located in 2007.

2.2. Data obtaining

2.2.1. Establishment of a coordinate (*x*, *y*) system

With the purpose of recording the position of the nests in the most accurate possible way, an observation grid was installed in the nesting area, which was composed of two perpendicular axes (*x*, *y*) and a coordinate origin (0, 0).

Figure 6. General view of the plot where the nesting areas of Almarail (Soria, Spain) were located. Picture by Y. Ballesteros.

Figure 7. Nesting area of *Stizus continuus* at "La Mallada Larga" (Valencia, Spain). Picture by J. D. Asís.

This coordinate system was installed every year, at the beginning of the flying season, and remained until the end of the observations, in the late summer, when it was withdrawn. In the (0, 0) point, as well as every 10 m along the x and y axes, markers were placed to help in nest position recording (Fig. 8). The area of the grid varied slightly through the different years, depending on the degree of nest aggregation, as well as on the number of nesting wasps. The position of the study area in Almarail (Soria) changed in the year 2010 relative to its position in 2008 and 2009 (Fig. 4), being established *ca.* 100 m to the southeast with respect to the previous years. However, the plot where the aggregations were located offered homogeneous conditions, in such a way that the different nesting areas presented similar characteristics.

2.2.2. Marking of wasps and measuring of biometric parameters

Every female wasp belonging to the studied species detected on the nesting area was captured for her marking, measurement and weighing, and was afterwards released. Wasps were located visually and/or acoustically (as they produce a typical buzzing sound), and captured in flight while they were feeding on flowers, or when they entered or went out of their nests, using an entomological net. The marking was necessary to track the individual wasps. Each of the wasps was marked with an individual combination of 3 colour dots on the thorax, using Uni Paint Mitsubishi ™ Markers, a well established technique in behavioural studies with wasps (Alexander and Asís, 1997; Asís *et al*., 2004; Polidori *et al*., 2006a, b, 2008, 2010a; Ballesteros *et al*., 2012). Additionally, the head width of each individual was measured with a digital caliper (±0.01 mm). Head width is a parameter commonly employed as an estimator of wasp body size (O'Neill, 1983; O'Neill and Evans, 1983; Asís *et al*., 1996). Finally, the wasp was weighed on an Ohaus Scout Pro Scales (±0.001 g), enclosing it in a previously weighed 2-ml Eppendorf vial to restrict its movements, and being then released.

2.2.3. Nest marking and positioning

Nests were marked utilizing stones, sticks or other natural materials, in such a way that they were easily visible to the observers (Fig. 9). Those objects were marked with the same colour code as the thorax of the owner of the nest. The marking system provided information about the position of the nest entrance and the direction in which the burrow had been built, as well as about the nest condition (active *vs*. completed).

Figure 8. Nesting area of Almarail (Soria, Spain). Some of the nests (in yellow) are appreciated, as well as one of the markers of the coordinate (*x*, *y*) system (in red). Picture by Y. Ballesteros.

Figure 9. Marked *Bembix* nest. The vertical stick shows the colour code of the female which owns the nest. Picture by Y. Ballesteros.

2.2.4. Prey collection and measuring of biometric parameters

Prey from the three *Bembix* species and from *Stizus continuus* were obtained from the female wasps when they went back to their nests after the provisioning flights. The female, carrying her paralyzed prey, was captured with an entomological net, and released after she had dropped her prey, that fell to the bottom of the net.

Prey were weighed, and the time of capture and the colour code of the female that had captured them, registered. Prey were then killed with ethyl acetate vapours, and then pinned in entomological boxes in the case of the prey of *Bembix* (adults of Diptera), or preserved in ethanol (95%) in the case of the prey of *S. continuus* (nymphs and adults of Orthoptera), until their identification in the laboratory. Whenever possible, prey were identified to species level or, when it was not possible, identified to family level and separated into morphospecies.

Some of the studies proposed in the present investigation required the knowledge of the prey (Diptera) availability in the surrounding environment. To estimate the fly availability, each hour between 11:00 and 18:00, for 12-16 days every flying season, 5-minute radial transects were performed from the center of the nesting area in random directions (in sectors of 45°) to a maximum distance of 300 m, collecting all the dipterans detected. The maximum distance of 300 m was determined attending to previous observations, in which marked females nesting in the area had been seen at a maximum distance of 300 m from the nesting area. The part of the day when the radial transects were done was established attending to previous studies (Asís *et al*., 2004), which showed that about 95% of the prey provisioned by the female wasps were hunted within that period. Dipterans were captured in flight, perched on the ground or on plants, and were weighed and then released. Some specimens of each species or morphospecies were killed and pinned for later determination.

2.2.5. Data analyses and specific methods

The package XlStat 2012 (Addinsoft) was used to carry out the most statistical tests. In the following paragraphs, the different analyses performed in each chapter are briefly described, as well as the specific protocols and/or the additional programs utilized to perform the necessary analyses of some of them.

In Chapter 1, the maximum lift capacity was calculated attending to the thorax weight/body weight ratio reported by Coelho *et al*. (2008) for the species *Bembix troglodytes*, for which (following Marden, 1987) they estimated the flight muscle weight as 95% of the thorax mass, and thus an *FMR* (*Flight Muscle Ratio*) of 0.36. Marden (1987) had previously defined the flight muscle ratio (*FMR*) from the flight muscle weight (*FMR*= flight muscle weight/body weight), and had observed that in bees and wasps the minimum *FMR* value that would allow the takeoff (called *marginal FMR* by Marden) was 0.179. This ratio would, therefore, be what would limit the additional weight (e.g. a prey) that a wasp could transport in flight (as well as its own weight) and is what was used here to assess whether the females transported prey with weights close to the theoretical maximum in order to optimize their provisioning flights. To assess if the frequencies of capture of the different prey families were similar to prey availability in the environment, Chi-square tests were done, considering as expected frequencies those found in the samplings performed in the area. To look for differences between the prey frequencies of the different weight classes and their availability in the environment, the standardized residuals were used:

$$SR = \frac{(observed - expected)}{\sqrt{expected}},$$

determining their significance with the critical value of the *Z*-distribution. The potential existence of a correlation between the weight of each female and the weight of the largest prey captured by her was assessed with a Pearson correlation test. To calculate the differences among weights for the different groups of flies (wasp prey *vs*. flies from the surroundings; weights of the different dipteran families), Student's *t*-tests were used, employing the Welch-Satterthwaite method when the variances were not homogeneous. The comparison of the weight of the largest prey captured by each female with the theoretical maximum load that the female can support in flight (considering a marginal *FMR* of 0.179) was done with a paired *t*-test.

In Chapter 2, the *FMR* for the different *Bembix* and dipteran species was calculated experimentally from the sample, and the *Wing Loading* (*WL*) was calculated as the ratio of total body mass (g) to total wing area (cm^2). The theoretical maximum load that a wasp is able to carry in flight was estimated with the following expression provided by Marden (1987):

$$M_{Pmax} = \frac{Log(M_{fm} - 1.01 + 1.76)}{9.80665} - M_w,$$

where M_{fm} is the flight muscle mass and M_w is the wasp mass. The margin between the M_{Pmax} and the heaviest prey actually captured by each female was here called *DL*, and calculated as:

$$DL = \frac{M_P}{M_{Pmax}} x\ 100,$$

where M_p is the mass of the largest prey actually captured by a wasp. ANOVAs (with post-hoc Tukey HSD-tests) were used to detect *FMR* or *WL* differences in the overall prey captured by both *Bembix* and those present in the environment. ANCOVAs were conducted to assess a series of dependent variables separately (mean *FMR* or *WL* of the prey captured by individual females, heaviest prey or *DL* of individual females, as well as own wasps' morphological traits (wing area, *WL*, *FMR* and thorax mass)) (dependent~ wasp mass; wasp species; wasp mass*wasp species), and maximal models were simplified by manual stepwise backward procedures, where only factors with $P < 0.05$, or those included in an interaction with $P < 0.05$, were kept in the model.

In Chapter 3, intra-specific overlap in prey taxa was calculated as:

$$PS_i = 1 - 0.5\ \sum_j \left| p_{ij} - q_j \right|,$$

(Bolnick *et al.*, 2002), where p_{ij} is the proportion of the *j*th prey species in individual *i*'s diet, and q_j is the proportion of the *j*th prey species in the whole species' prey spectrum. The mean value of the PS_i (called *IS*) expresses the average individual specialization for a species, and was subtracted to 1 to obtain the more intuitive index *V*. Intra-specific overlap in prey weight was calculated with Roughgarden's Index (*R'sI*) (Roughgarden, 1974) for continuous data (Bolnick *et al.*, 2002):

$$R'sI = \frac{WIC}{TNW} = \frac{E\left[Var(x_{ij}|i)\right]}{Var(x_{ij})},$$

where *WIC* represents the within-individual component, and *BIC* represents the between-individual component, of the total niche width (*TNW*), and x_{ij} is the weight of the *j*th prey item in individual *i*'s diet. The more intuitive *W* index was obtained by subtracting *R'sI* to 1. Both *V* and *W* vary between 0 and 1, with values near 1 indicating higher individual specialization. Both PS_i and *R'sI* were calculated with the program IndSpec1 (Bolnick *et al.*, 2002). The pairwise taxonomic diet dissimilarity (the complement of the diet similarity *PS*, Bolnick and Paull, 2009) between each pair of individuals *i* and *j* was calculated as:

$$PDS_{ij} = 1 - \sum_{k=1}^{n} min\left(p_{ik}, p_{jk}\right),$$

where p_{ik} and p_{jk} are the proportions of the *k*th prey type in individual *i*'s and *j*'s diet, respectively (Ingram *et al.*, 2011). The mean value of PDS_{ij} (called E_T) represents the overall level of prey taxa variation within the population (Ingram *et al.*, 2011). The calculation of the pairwise prey size dissimilarity (SDS_{ij}) between each pair of individuals *i* and *j* was done with the following formula developed for the present study:

$$SDS_{ij} = \sqrt{\left| \frac{(Pm_i - Pm_j) + (Pcv_i - Pcv_j)}{Pm_i + Pm_j} \right|},$$

where *Pm* is the mean prey size of the individual and *Pcv* is the coefficient of variation in the prey size of the individual. Dissimilarity matrices for prey taxa (*PDS*) and for prey size (*SDS*) were built from the PDS_{ij} and SDS_{ij} values. From the body mass of the wasps, another matrix (the matrix *Wasp_MASS*), based on the pairwise difference of body mass between females was built, calculating the paired size differences as:

$$\left|mass_i - mass_j\right| / max\left(mass_{ij}\right)$$

An additional matrix was obtained from the distances between each nest and all the others (*ND*) (*NEST* matrix). *Wasp_MASS* matrix was plotted against *PDS* matrix and *SDS* matrix with Mantel tests to look for correlations between the former and any of the other matrices. A significant correlation would mean that body size difference affects the level of prey (taxa and/or size) dissimilarity. *NEST* matrix was plotted against *PDS* matrix with a Mantel test. In this case, if pairwise inter-nest distance explains the degree of prey taxa dissimilarity, the two matrices would be correlated.

In Chapter 4, inter-individual variation in resource use was assessed, for both prey species and sizes, through the index *E* (Araújo *et al.*, 2008). The index will approach 0 when diets are similar among individuals, and 1 for maximum variation (Araújo *et al.*, 2010). The search for different patterns of individual resource use was performed (for both prey species and sizes) by utilizing different indices (C_{ws}, *NODF*, *C-Score*) and programs. The existence of clustering in the population was studied by means of the C_{ws} index for the relative degree of clustering (Araújo *et al.*, 2008), which varies between -1 (indicating overdispersion) and +1 (denoting clustering) (Araújo *et al.*, 2010). Both *E* and C_{ws} were calculated with the program Dieta1 (Araújo *et al.*, 2008). The binary matrices from Dieta1 were imported into the program Pajek (Batagelj and Mrvar, 1998) to visualize the dietary distribution of the females. The degree of nestedness was analyzed with the index *NODF* (Almeida-Neto *et al.*, 2008) in the program ANINHADO 3.0 (Guimarães and Guimarães, 2006). *NODF* will equal 0 for the absence of nestedness and 100 for maximum nestedness. The *C-Score* index (Stone and Roberts, 1990) was employed to look for checkerboard patterns, utilizing NODF-Program 2.0 (Almeida-Neto and Ulrich, 2011). *C-Score* varies between 0 (for no checkerboards) and 1 (for a perfect checkerboard pattern). Additionally, when clusters were found, ANOVAs (with post-hoc Tukey HSD-tests) or Student's *t*-tests were performed to check whether they were constituted on the basis of the different body masses of the females, to test for a possible effect of the simultaneity in time of the females belonging to different clusters and to compare the mean distances between pairs of nests belonging to the same cluster with the mean distances between pairs belonging to different clusters. When nestedness was found, linear regressions were performed to test whether wasp body mass was related to the percentage of different species or size groups captured (taking as 100% the total number of prey species or size categories), and Pearson correlation tests were done to ascertain if the number of wasps preying upon a particular prey species or size group depended on its availability in the surroundings.

In Chapter 5, the *K* distribution (Ripley, 1976) was employed to analyse the spatial distribution of the nests, utilizing the Duncan's (1991) program. The results obtained were transformed following the function $L(t)= (K(t)/\pi)^{1/2}- t$, where t is the spatial step (scale) with which the test was performed (Haase, 1995). A clumped pattern would mean conspecific attraction during nest establishment. Also, a goodness-of-fit analysis was done to evaluate if the presence of conspecific females excavating new nests could influence a female's decision to begin a nest at a particular spot, determining if the number of new nests simultaneously dug in the same 3 x 3 m square (in one on the years of the study, a grid composed by 30 squares of 3 x 3 m each had been established in the field, monitoring the number of females simultaneously digging new nests) fitted a binomial-negative distribution (Quinn and Keough, 2002). To assess whether the females tended to establish new nests close to the one previously finished, the distance between the new and the finished nest of each female for whom the positions of 2 or more consecutive nests were known was measured, comparing the observed distances with the mean distances obtained by 1,000 random simulations (considering as the maximum possible distance the one measured from the nest previously closed by the female to the farthest nest observed in the aggregation) with a Student's paired *t*-test, which was also used to compare the observed previous-new nest distances for a given female with the NND (nearest neighbour distance) to conspecific nests. If wasps prefer to dig a new nest close to the previous one, the observed previous-new nest distances would be smaller than the simulated nest-nest distances and smaller than NNDs. Minimum convex polygons of nest clusters were built in 2010 and inter-specific and intra-specific distances between their centroids were compared with a Student's *t*-test. Also NNDs between females within a species were compared with those between females of different species (Student's *t*-test). If heterospecific nests play a role in nest settlement decision, the distances among species would be either smaller (attraction) or larger (repulsion) than the distances within species.

In Chapter 6, we used a well-established method for the study of behavioural interactions in fossorial Hymenoptera (e.g. Boesi *et al*., 2009) in controlled conditions, the so-called *circle-tube* test, to analyse interactions between the different species of *Bembix* and *Parnopes* cuckoo wasps. The *circle tube* tests were carried out placing in a 45 cm-length, transparent silicon tube, combinations of a female wasp (*B. merceti*, *B. sinuata* or *B. zonata*) plus a female of a chrysidid (*P. grandior* or *H. longicolle*), and monitoring the response of the wasp towards the chrysidid (aggressive, tolerant or avoidance response). Then, the possible difference in the number of events per minute for each of the three responses occurring in each combination was evaluated with non-parametric, Kruskall-Wallis analyses (post-hoc Dunn tests for pairwise comparisons). To perform the chemical analyses of CHCs, we first processed the extracts of the three different *Bembix* species (*B. merceti*, *B. zonata* and *B. sinuata*) and two chrysidids (*Parnopes grandior* and *Hedychrum longicolle*) with Gas-Chromatography-Mass-Spectrometry (GC-MS) at the University of Freiburg (Germany); then, the obtained chromatogram peaks were integrated with the software Chem Station (Agilent Technologies, Böblingen, Germany), and their relative areas were transformed as:

$$log_{10}\left(\left(\frac{relative\ peak\ area}{geometric\ mean\ of\ the\ relative\ peak\ area}\right)+1\right),$$

following Strohm *et al*. (2008). A similarity analysis (ANOSIM), utilizing the Bray-Curtis distances, was performed to check whether species and sexes could be correctly split on the basis of their CHCs. Three additional ANOSIMs (Bray-Curtis distances) were done with the alkanes only, the alkenes only, and (after a preliminary multidimensional scaling graph, MDS, which allowed the *a priori* assignation of individuals to groups, and where a great proximity of *B. merceti* and *P. grandior* was observed) considering males of *B. merceti* and *P. grandior* as one group as well as females of those species as another group. From the total peak area, the number of nanograms per mg of body mass was calculated utilizing the known amount of standard previously added. Additionally, a cluster was elaborated. A Kruskall-Wallis analysis (post-hoc Dunn tests for pairwise comparisons) was performed to look for differences in the total amount of CHCs (normalized by body mass) in the females of the different species (to detect a possible strategy of insignificance). The MDS, ANOSIMs and cluster analysis were performed with PRIMER v.5 (Primer-Ltd).

OBJECTIVES

First.- Test the *ODT* predictions in a predatory insect that performs a progressive type of provisioning, analysing whether the female wasps try to optimize their provisioning flights by means of the capture of larger prey by bigger females, as well as through the capture of prey whose body mass is near the maximum load that each wasp is able to efficiently transport in flight.

Second.- Ascertain if female wasps carry out any kind of selection at the species level, based either on the taxa or on the weight of the prey captured, or, alternatively, they select their prey on the basis of their environmental availability (generalist wasps).

Third.- Study if the main morphometric traits associated to flight ability (body mass, *FMR* and *WL*) of both wasps and prey have any influence on resource selection patterns or on resource partition, at inter-specific (among sympatric species of the genus) as well at intra-specific level.

Fourth.- Assess the presence and strength (as well as the temporal variation) of individual specialization in different species of digger wasps, a pattern to date much more investigated and reported in vertebrates.

Fifth.- Find out the factors accounting for the observed degree of individual specialization in the studied digger wasps (in the cases where individual variation has been detected), through the assessment of the morphological distance between females, the distance between their nests and of network-theory derived indices.

Sixth.- Analyse the kind of spatial pattern (grouped, regular or random) exhibited by the nests included within wasp aggregations made up of sympatric species of digger wasps, and elucidate the causes (biotic factors) accounting for the establishment of the observed patterns.

Seventh.- Describe the composition of cuticular hydrocarbons (CHCs) profiles in different species of digger wasps, as well as in their potential parasitoid, the generalist chrysidid *Parnopes grandior*, in order to assess which kind of strategy (chemical mimicry, chemical camouflage or chemical insignificance) *P. grandior* utilizes to successfully exert its parasitism upon their different host species.

CHAPTER 1

Are solitary progressive-provisioning wasps optimal foragers? A study with the digger wasp *Bembix merceti* (Hymenoptera: Crabronidae)

Bembix merceti is a *central-place forager* that captures dipterans to feed its larvae. First, a test to find out whether the females behaved as generalists (i.e. to test if a concordance between the frequencies of hunted and available prey families and sizes existed) was performed. Second, the potential optimization in the capture of prey was evaluated in this species, in the framework of the *ODT*. The calculation of the maximum lift capacity of the female wasps, necessary to assess whether foraging optimization is taking place, was performed attending to the limitations imposed by the *Flight Muscle Ratio* (*FMR*).

B. merceti females do not behave as generalists while hunting flies, capturing prey families and sizes in proportions different from those present in the surrounding environment. Furthermore, they tend to optimize their captures, making a positive selection of fly taxa with greater mean weights, even though they are less abundant. Within the fly families whose mean weight is not too high, the females capture those prey whose weight is larger than the mean (Table 1). Thus, selection seems to be based on prey size, and not on the type (family) to which the prey belongs.

A significant correlation between the weight of each female and that of the largest prey captured by her is present (Fig. 1), suggesting that the females capture prey according to their load-lifting capacity.

Table 1. Prey captured by the females of *Bembix merceti* and dipterans available in the environment, with the mean weight per family (only families with at least 3 prey captured or with more than 2% of captures in the environment are shown).

	Environment		Prey		Residual significance	Mean weight of environmental Diptera (mg)	Mean prey weight (mg)	Mean weight significance
	%	N	%	N				
Syrphidae	75.8	141	60.4	110	(−) *P* = 0.02	14.0 ± 4.6	15.5 ± 4.5	t = 2.62 *P* = 0.009
Bombyliidae	8.1	15	34.1	62	(+) *P* = 0.001	38.1 ± 39.5	27.7 ± 20.1	t = −1.05 *P* = 0.310
Stratiomyidae	1.1	2	2.7	5	(+) *P* = 0.03	12.2 ± 4.2	17.0 ± 6.5	
Tabanidae	0.5	1	1.6	3	(+) *P* = 0.04	28.0	29.7 ± 5.8	
Sarcophagidae	4.8	9	0.5	1	-	12.7 ± 5.3	30.0	
Tachinidae	4.8	9	0	0	-	18.0 ± 5.0	-	
Culicidae	2.2	4	0	0	-	4.3 ± 1.9	-	
Others	2.7	5	0.5	1	-	11.8 ± 9.1	20.0	
Total	100	186	100	182		15.9 ± 13.6	20.1 ± 13.6	t = 2.96 *P* = 0.003

In the case of Syrphidae and Bombyliidae, and the total number of prey, the mean weight in the environment is compared with that of the prey. The (+, -) signs in the residual significance column reflect the capture of prey in frequencies larger (+) or smaller (-) than expected as a function of the availability. In the *Others* section, the families Anthomyidae, Asilidae, Calliphoridae, Chironomidae and Muscidae are included.

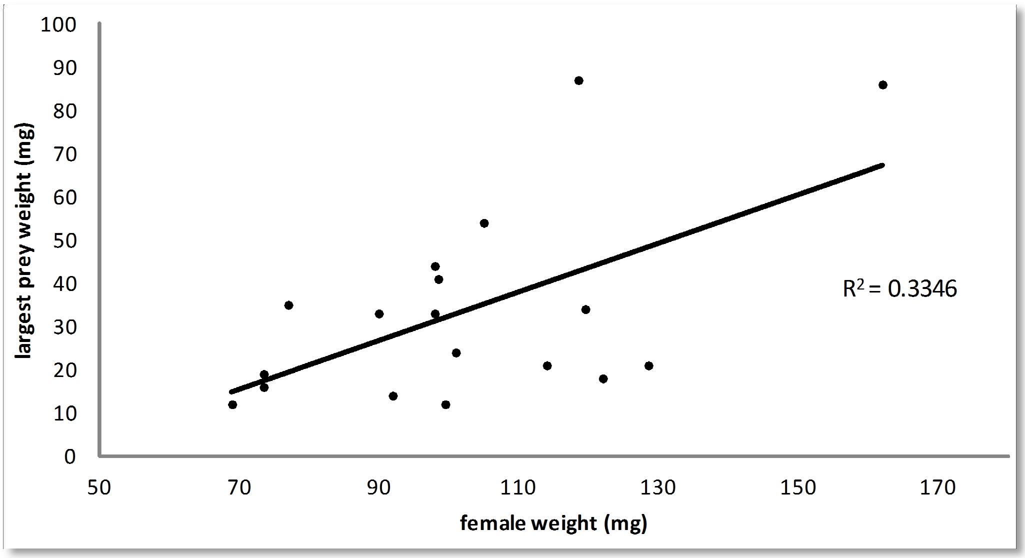

Figure 1. Body weight of the largest prey captured by each *Bembix merceti* female as a function of the weight of each female.

Nevertheless, captures were not optimized maximally; the females maintained a margin with respect to the maximum prey weights that they could transport efficiently (Fig. 2), in such a way that *B. merceti*, in general terms, can be considered a suboptimal forager. The existence of the abovementioned margin could be related to the low availability of large prey in the environment, to the type of progressive provisioning shown by the females of this species, and to other factors, such as the good manoeuvrability of their prey (in general, flies are rapid fliers) and the pressure from their natural enemies and congenerics (if females take smaller prey, they will be able to enter their nests more rapidly, reducing the risk of parasitism and predation).

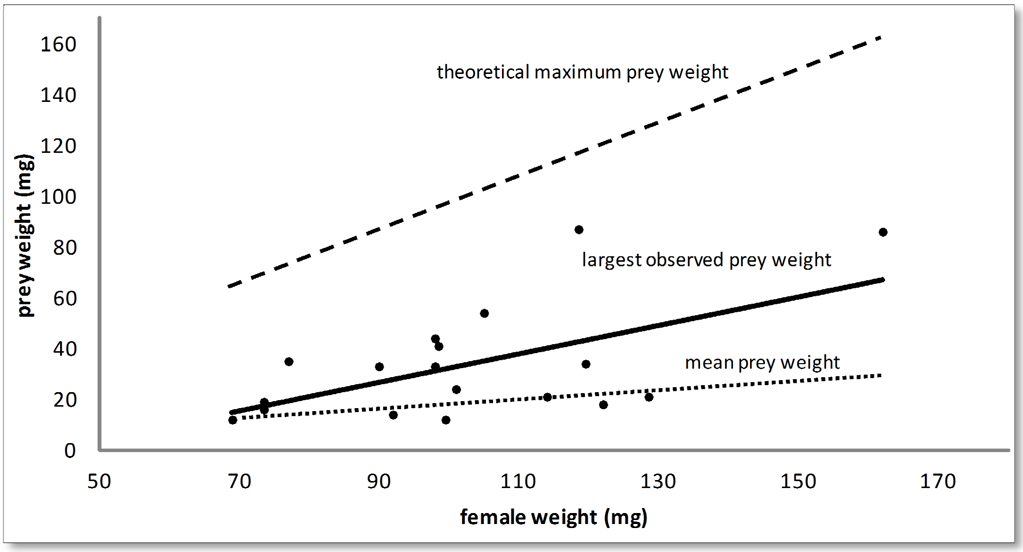

Figure 2. Relationships between *Bembix merceti* female body weight and: the theoretical maximum prey weight, considering a marginal FMR of 0.179 (the theoretical maximum weight which females are able to lift (Marden, 1987)) (dashed line); the regression line obtained with the weight of the largest prey captured by each female (solid line); and the regression line obtained with the mean prey weight for each female (dotted line). The values obtained for the weight of each female and that of the largest prey captured by each female are also provided.

CHAPTER 2

Is individual prey selection mediated by flight ability-related morphology of prey in *Bembix* digger wasps?

Flight-associated morphometric traits, such as the ratio of flight muscle mass to body mass (*Flight Muscle Ratio*, *FMR*) and the ratio of body mass to wing area (*Wing Loading*, *WL*) are known to affect load-lifting capacity, manoeuvrability and other parameters related to flight ability. In predator-prey interactions in which the interacting organisms are both fliers, these traits could be under selection to maximize predator hunting success and prey escape ability.

Here it was analysed the flight-related morphology of the digger wasps *Bembix merceti* and *B. zonata* and their targeted prey (Diptera). Wasp *FMR* resulted to be species-specific and higher in *B. zonata* (Table 2), not varying with wasp mass, resulting in a greater capacity of this species to carry larger prey in flight.

Table 2. Mean values (± SD) for the different biometric parameters and indices measured for wasps and prey. The sample size employed in each case for *B. merceti* and *B. zonata* is indicated in the fourth and fifth columns.

	B. merceti	***B. zonata***	**N *(B. merceti)***	**N *(B. zonata)***
Heaviest prey individual wasps (g)	0.032 ± 0.020	0.048 ± 0.029	34	21
Predicted maximum load (g)	0.055 ± 0.122	0.129 ± 0.167	34	21
Mean DL	30.303 ± 14.776	15.860 ± 5.899	34	21
Wasp body mass (g)	0.102 ± 0.020	0.115 ± 0.017	146	61
Wasp thorax mass (g)	0.030 ± 0.008	0.055 ± 0.008	10	7
Wasp FMR	0.306 ± 0.037	0.430 ± 0.032	10	7
Mean FMR of the prey (individual wasps)	0.343 ± 0.035	0.377 ± 0.057	34	21
Mean FMR of the prey (overall)	0.342 ± 0.082	0.383 ± 0.079	413	212
Wasp wing area (cm^2)	0.515 ± 0.041	0.591 ± 0.138	10	7
Wasp WL (g·cm^{-2})	0.184 ± 0.051	0.214 ± 0.039	10	7
Mean WL of the prey (individual wasps) (g·cm^{-2})	0.060 ± 0.019	0.055 ± 0.007	34	21
Mean WL of the prey (overall) (g·cm^{-2})	0.061 ± 0.040	0.054 ± 0.017	413	212

The *WL* of both *Bembix*, on the contrary, varied (directly) with the mass of the wasps, not diverging between species (Table 2), due to a static size of the wings. Maximum theoretical load was much higher than the actual prey load in the two *Bembix* species, with greater distance to their maximum load (lower *DL*) in *B. zonata* (Table 2), perhaps due to the greater parasitic pressure exerted by the chrysidid wasp *Parnopes grandior* on this species compared to *B. merceti*. As wasp mass increased, the heaviest prey captured and the mean prey *FMR* increased in *B. merceti*, but decreased in *B. zonata* (Figs. 3 and 4).

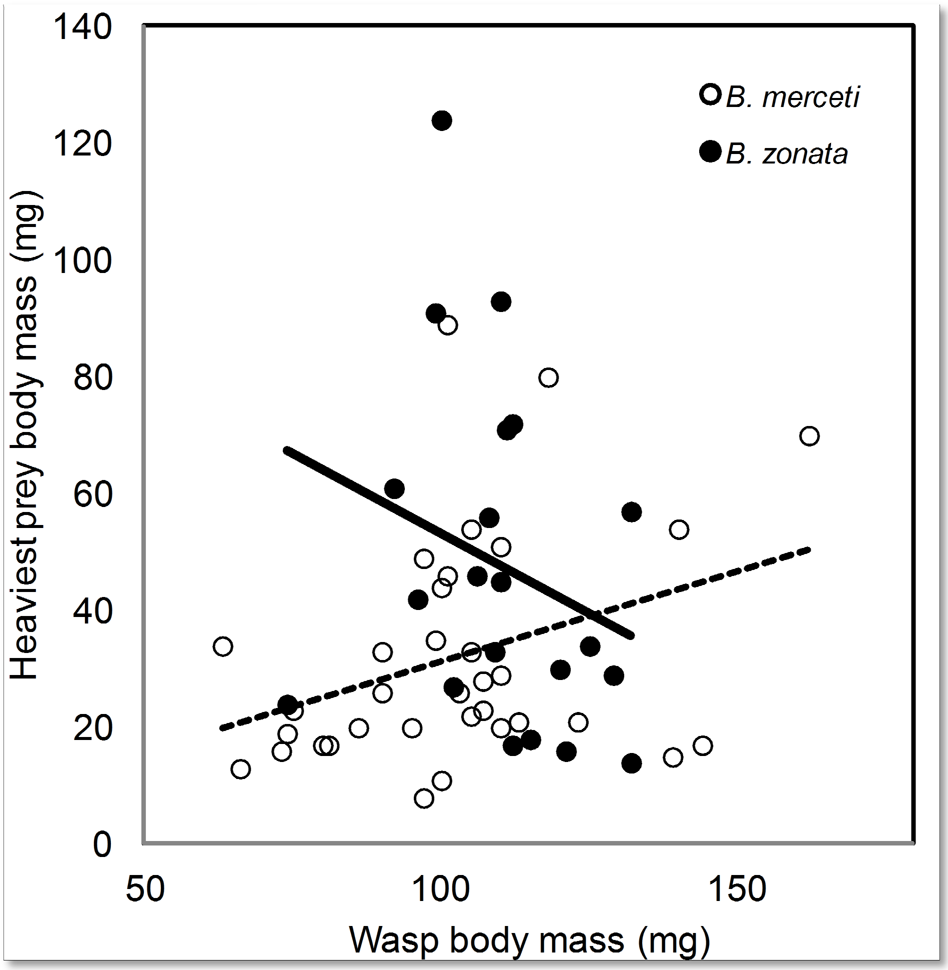

Figure 3. GLM plot of the weight of the heaviest prey captured by both *Bembix* species as a function of wasp's mass. Dotted line: trend line for *B. merceti*; continuous line: trend line for *B. zonata*.

Overall, *B. zonata* prey had lower mean *WL* and higher mean *FMR* than *B. merceti* prey (Table 2), possibly according to the size difference between both wasp species (Table 2). Thus, individual-based prey selection seems to be at least partially affected by flight ability-related morphology of prey in *Bembix* digger wasps.

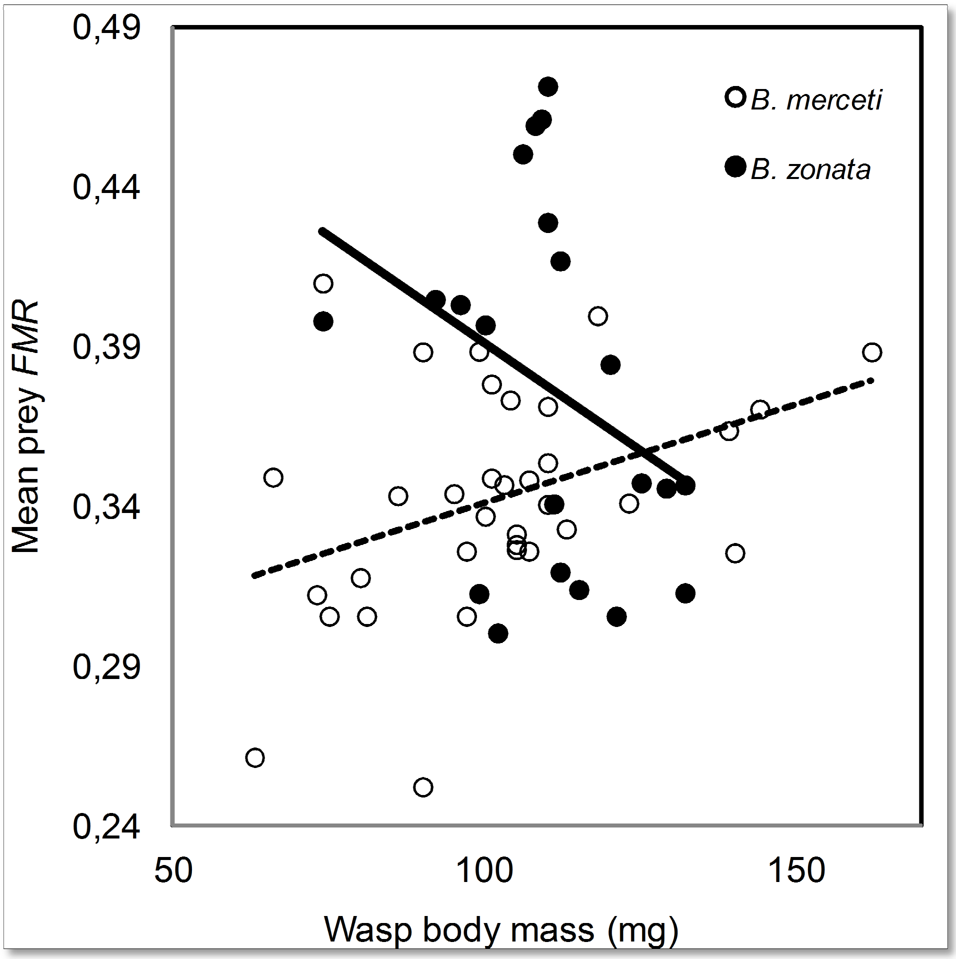

Figure 4. GLM plot of the mean FMR of the prey captured by both *Bembix* species as a function of wasp's mass. Dotted line: trend line for *B. merceti*; continuous line: trend line for *B. zonata*.

CHAPTER 3

Morphological distance and inter-nest distance account for intra-specific prey overlap in digger wasps (Hymenoptera: Crabronidae)

Although inter-individual diet variation is common in predatory wasp populations, the factors accounting for such variation are still largely unknown. In the present chapter, it was assessed whether paired diet dissimilarity in prey use correlates with morphological distance (differences in body size) and/or with inter-nest distance in three species of digger wasps: *Bembix merceti*, *Bembix zonata* and *Stizus continuus*. Morphological distance and inter-nest distance have been previously found to be linked to diet partitioning in vertebrates.

Results were heterogeneous, varying among species and generations. On one hand, all sampled populations displayed significant inter-individual diet variation for prey taxa; on the other hand, only in the half of the cases inter-individual variation for prey size was found (Table 3).

In one of the generations of *B. zonata* and *S. continuus*, wasps of similar size had similar prey taxonomic spectra (Figs. 5-c and 5-a) (in the case of *S. continuus*, also their prey size spectra were similar, Fig. 5-b), a phenomenon which probably reduces intra-specific competition.

Additionally, in one of the years, the females of *B. zonata* that nested closer to each other had more similar prey taxonomic spectra (Fig. 5-d), suggesting that distant females probably hunt on different patches that harbour different prey species.

Table 3. Individual diet specialization and pairwise diet dissimilarity between females for each species/period studied.

Especies and period	**Individual specialization for prey taxa (*V*)**	**Individual specialization for prey size (*W*)**	**Pairwise prey taxonomic dissimilarity**	**Pairwise prey size dissimilarity**
B. merceti 2009	0.531 ± 0.202, $P < 0{,}0001$	0.164, NS	$PDS_{ij} = 0.246 - 1$ $E_T = 0.704 \pm 0.253$	$SDS_{ij} = 0.094 - 0.807$ $E_S = 0.395 \pm 0.197$
B. merceti 2010	0.412 ± 0.178, $P < 0.0013$	0.384, $P < 0.0001$	$PDS_{ij} = 0.142 - 1$ $E_T = 0.621 \pm 0.344$	$SDS_{ij} = 0.040 - 0.756$ $E_S = 0.426 \pm 0.162$
B. zonata 2009	0.514 ± 0.185, $P < 0.0001$	0.174, NS	$PDS_{ij} = 0.142 - 1$ $E_T = 0.654 \pm 0.283$	$SDS_{ij} = 0.054 - 0.719$ $E_S = 0.384 \pm 0.154$
B. zonata 2010	0.391 ± 0.127, $P < 0.0049$	0.066, NS	$PDS_{ij} = 0.076 - 0.75$ $E_T = 0.460 \pm 0.196$	$SDS_{ij} = 0.149 - 0.664$ $E_S = 0.436 \pm 0.155$
S. continuus 2007-I	0.336 ± 0.259, $P < 0.0001$	0.341, $P < 0.0001$	$PDS_{ij} = 0 - 1$ $E_T = 0.389 \pm 0.423$	$SDS_{ij} = 0.007 - 0.801$ $E_S = 0.352 \pm 0.221$
S. continuus 2007-II	0.567 ± 0.152, $P < 0.0001$	0.494, $P < 0.0001$	$PDS_{ij} = 0 - 1$ $E_T = 0.695 \pm 0.315$	$SDS_{ij} = 0.041 - 0.756$ $E_S = 0.447 \pm 0.169$

For the two last columns, min-max range precedes mean ± SD. NS means “non-significant”.

In the case of the females of *B. merceti*, neither differences in wasp size nor inter-nest distance affected prey dissimilarity.

Both morphological distance and inter-nest distance are potentially important in shaping the overlap of individual resource use in wasps, although probably only under certain conditions, such as highly clumped distribution of nests and the existence of size-related constraints on prey selection.

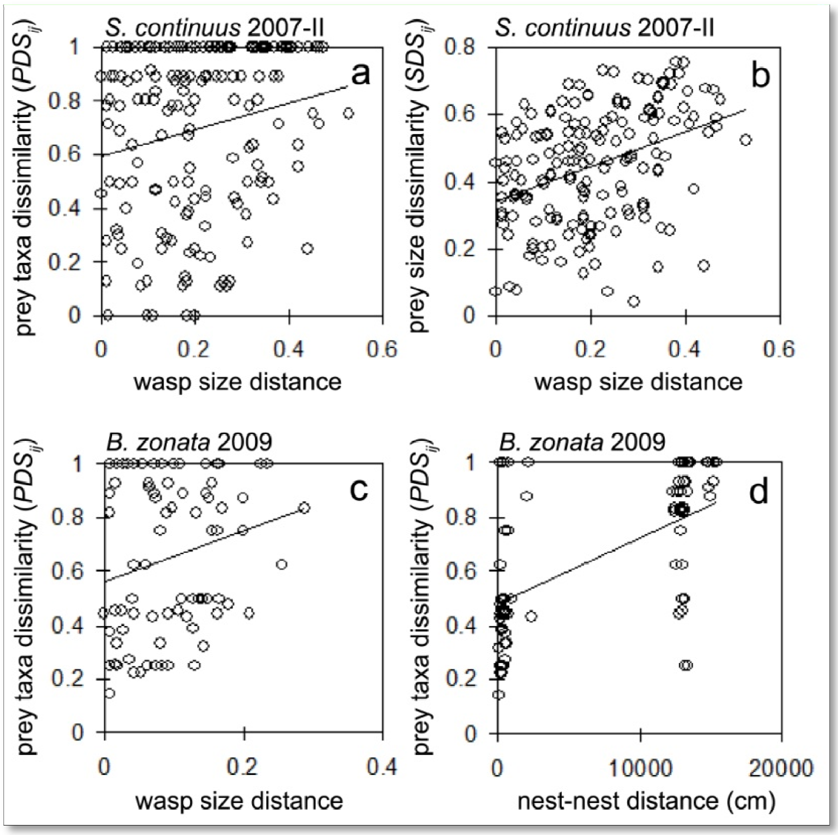

Figure 5. Significant relationships observed. **a**. Between pairwise wasp size distance and pairwise prey taxa dissimilarity of *Stizus continuus* 2007-II (P= 0.008). **b**. Between pairwise wasp size distance and pairwise prey size dissimilarity of *S. continuus* 2007-II (P< 0.0001). **c**. Between pairwise wasp size distance and pairwise prey taxa dissimilarity of *Bembix zonata* 2009 (P= 0.055). **d**. Between pairwise inter-nest distance and pairwise prey taxa dissimilarity of *B. zonata* 2009 (P< 0.0001), as calculated with a two-tailed Mantel test. The linear least squares lines for the observed regressions of diet dissimilarity functions (PDS_{ij} y SDS_{ij}) are shown. Note that the scales of both vertical and horizontal axes differ among panels.

CHAPTER 4

Patterns of inter-individual diet variation in *Bembix merceti* (Hymenoptera: Crabronidae): a network-based approach

Energy obtaining is paramount for the survival of organisms, and the study of the factors shaping their trophic niches has been the aim of large numbers of works. Although diet has traditionally been considered as a property of the species or populations as a whole, there is nowadays extensive knowledge that individual specialization is widespread in animal world, and that populations that behave as generalists that consume a wide range of resources, may be in turn composed of individual specialists that consume small subsets of the population's niche. Nevertheless, individual specialization has been little quantified among wasps.

In a population of the digger wasp *Bembix merceti*, the intra-specific variation in resource use for three consecutive flying seasons (2008, 2009 and 2010) was studied. Individual variation in the selection of both prey species and sizes was assessed through the utilization of network-based indices.

Regarding the species of prey captured, inter-individual variation was found in all three years (Table 4-1), and could be due to the acquisition of particular hunting strategies by individual wasps. Females constituted dietary clusters in 2009 (Table 4-1, Fig. 6), which were not organized on the basis of the wasps' body mass or temporal simultaneity, or of the spatial distance among their nests. Females originated nested patterns in 2008 and 2010 (Table 5-1), where larger females consumed a higher proportion of the total number of prey species captured by their population, and more abundant prey species were present in the diet of a greater number of wasps.

Table 4. Values and significance of E, experimental C_{ws} values, and 97.5% confidence intervals of C_{ws}, for the captured species (4-1) and prey size groups (4-2). Significant values in bold.

	Species (Table 4-1)				Size groups (Table 4-2)			
Population	E	$P\ (E)$	C_{ws}	97.5% CI (C_{ws})	E	$P\ (E)$	C_{ws}	97.5% CI (C_{ws})
2008	0.772	**0.000**	0.099	0.061 - 0.179	0.648	**0.016**	**0.000**	0.074 - 0.190
2009	0.762	**0.000**	**0.182**	0.034 - 0.139	0.600	**0.000**	**-0.038**	0.024 - 0.107
2010	0.672	< **0.001**	0.102	0.027 - 0.144	0.452	0.742	0.057	0.023 - 0.108

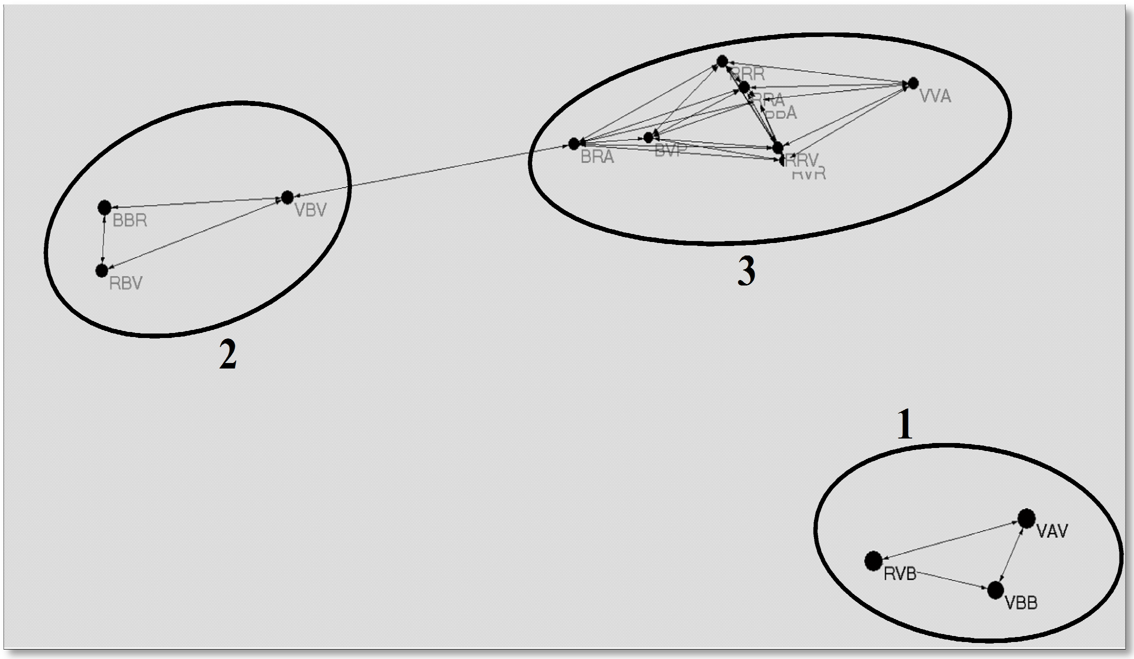

Figure 6. Spatial representation of the 2009 females performed with Pajek (Batagelj and Mrvar, 1999). Three clusters (superimposed ellipses) appear, on the basis of similar or dissimilar captures among the individuals.

The checkerboard pattern detected in 2009 (Table 5-1, Fig. 7) suggests the idea that the observed inter-individual variation is produced by the acquisition of specific hunting strategies by individual females, as the development of certain hunting techniques could prevent females from using resources whose manipulation needs from different techniques.

Table 5. Experimental values of *NODF* with associated *P*-value, and *C-Scores* with associated *P*-values and 97.5% confidence intervals, for the captured species (5-1) and prey size groups (5-2) in each of the studied populations.

	Species (Table 5-1)					**Size groups (Table 5-2)**				
Population	*NODF*	*P* (*NODF*)	*C-Score*	*P* (*C-Score*)	97.5% CI (*C-Score*)	*NODF*	*P* (*NODF*)	*C-Score*	*P* (*C-Score*)	97.5% CI (*C-Score*)
2008	42.80	**0.010**	0.045	0.267	0.018 - 0.062	68.69	0.080	0.025	0.324	0.005 - 0.073
2009	29.15	0.110	0.048	**0.003**	0.014 - 0.041	63.47	0.980	0.067	**0.012**	0.006 - 0.061
2010	30.11	**0.020**	0.030	0.061	0.008 - 0.033	65.87	0.150	0.036	0.477	0.514 - 0.077

Regarding the selection of prey sizes, inter-individual variation was also commonly detected, being absent only in 2010 (Table 4-2), a fact that could obey to the increase in the population density observed on that year. Populations didn't exhibit clustering in any of the three years, but showed overdispersion in 2008 and 2009 (Table 4-2). This fact, together with the absence of inter-individual variation in 2010, could indicate the affiliation of the population to the *distinct preferences* model (Svanbäck and Bolnick, 2005). Nestedness of females with respect to prey size was absent in all three years (Table 5-2).

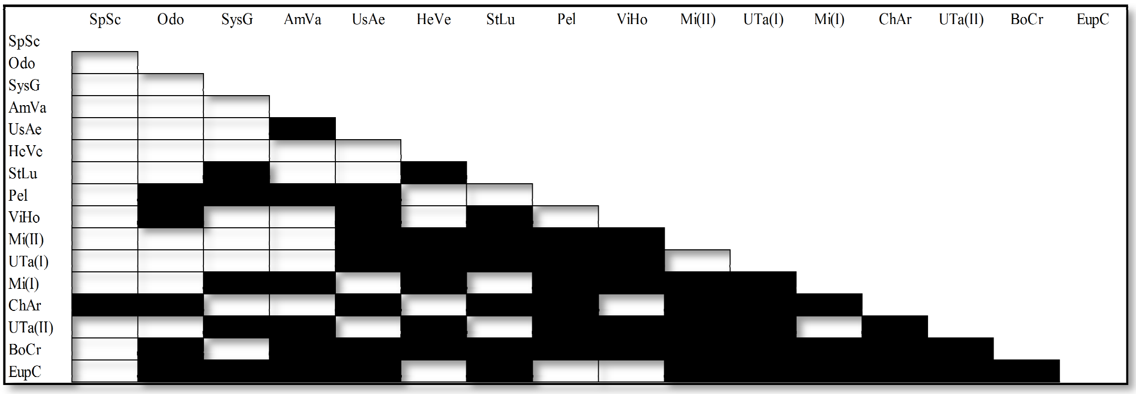

Figure 7. List of prey species never co-occurring together (black squares), or co-occurring at least once (white squares) within the pool of a given female wasp. SpSc= *Sphaerophoria scripta*; Odo= *Odontomyia* sp.; SysG= *Systoechus gradatus*; AmVa= *Amictus variegatus*; UsAe= *Usia aenea*; HeVe= *Hemipenthes velutinus*; StLu= *Stomorhina lunata*; Pel= *Peleteria* sp.; ViHo= *Villa hottentotta*; Mi(II)= Miltogramminae (II); UTa(I)= Unidentified Tachinidae (I); ChAr= *Chrysotoxum arcuatum*; UTa(II)= Unidentified Tachinidae (II); BoCr= *Bombylisoma croaticum*; EupC= *Eupeodes corollae*.

CHAPTER 5

Bembix digger wasps rely on conspecifics location and not on previous experience or heterospecifics presence during nest-settlement decisions

Compared to abiotic environmental factors, attraction or repulsion behaviours towards conspecifics or heterospecifics have been less investigated as driving forces for the spatial arrangement of individuals. Solitary females of digger wasps (Hymenoptera) often nest in dense aggregations, a characteristic that makes these insects good models for the assessment of spatial patterns. Here, using as a model a multi-specific nest aggregation of three species of the genus *Bembix*, it is assessed for the first time if wasp females are attracted to or repulsed by conspecific nests, heterospecific nests or their own previously established nests, when choosing their nest-digging locations.

A clumped pattern of nests was evident early in the season for each species (Fig. 8, left column), but later in the season a random distribution of nests was more common (Fig. 8, right column), suggesting the existence of conspecific attraction at the beginning of the nesting season. Such behaviour was confirmed by the fact that females initiated their nests more frequently where other females of their species were digging. Subsequent nests dug by individual females were closer to their previous nests than in random simulations (Table 6). However, this pattern seemed to depend on the tendency of wasps to dig close to conspecifics rather than remain in the vicinity of previous nests, suggesting that females' experience on future decisions matters only at a large scale.

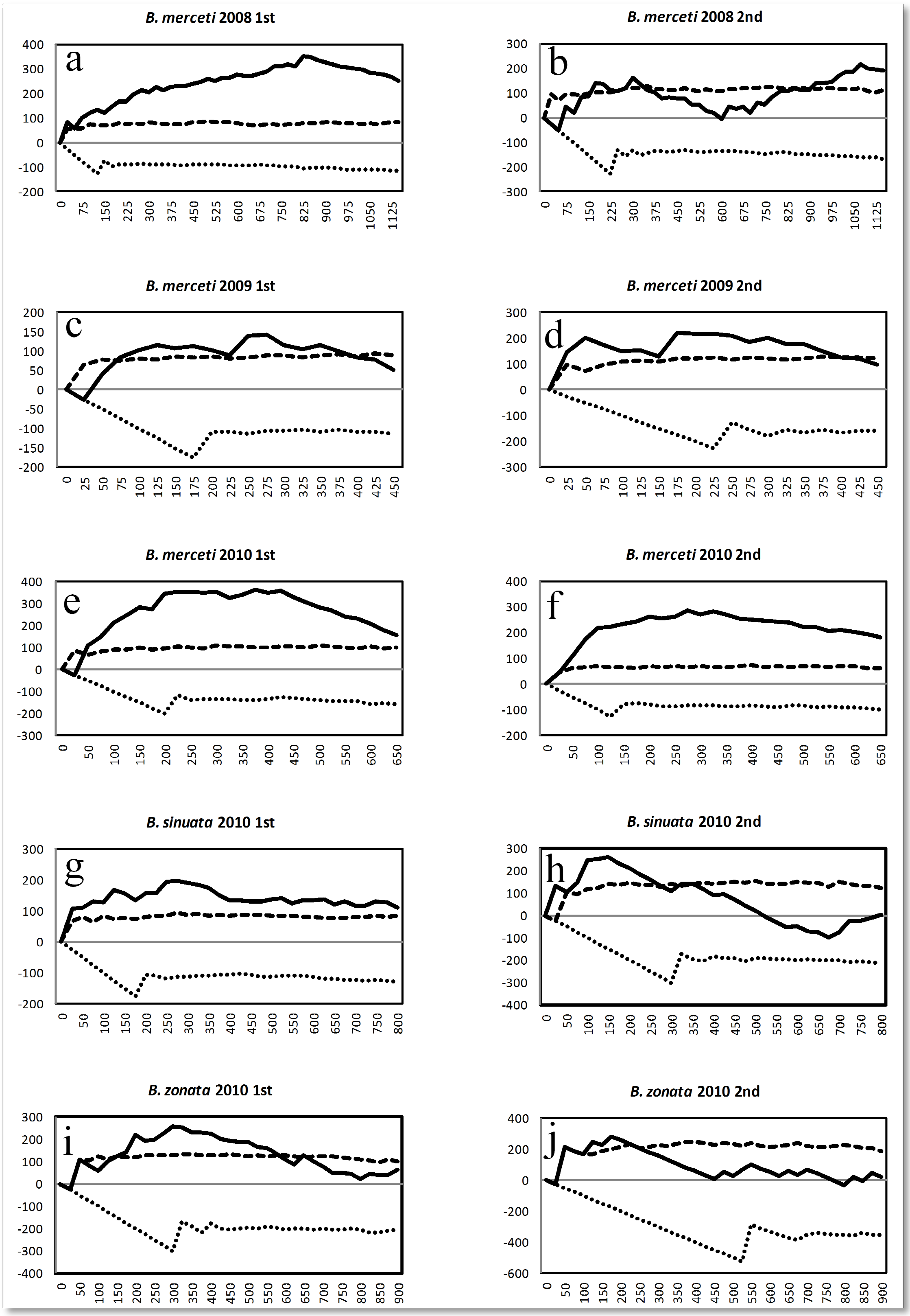

Figure 8. Ripley's *K* functions for the three species studied, separated by periods. Bold line: transformed Ripley's *K* function ($L(t)= (K(t)/\pi)^{1/2} - t$), calculated from observed data and for given spatial steps; dashed line: upper simulation envelope (limits for significance); dotted line: lower simulation envelope (limits for significance). Step used was 25 cm. Units of *x* axis are cm.

Table 6. Total number of females marked during the study, number of females with two or more nests observed in the aggregations, number of new nests established within different range distances from the previous nests, observed and simulated previous-new nest distances, and nearest neighbour distances (NND) of the new nests. The maximum distance is the one from the previous nest to the new one which could be reached by females if they didn't disperse outside the nest aggregation.

Species (year)	**% of females with ≥ 2 nests (total number of females)**	**Number of consecutive nests**	**Number of new nests at < 25% of maximum distance**	**Number of new nests at 26-50% of maximum distance**	**Number of new nests at 51-100% of maximum distance**	**Observed previous-new nest distance (cm)**	**Simulated previous-new nest distance (cm)**	**NND of the new nests (cm)**
B. merceti (2008)	48% (27)	16	8 (50%)	6 (38%)	2 (12%)	578 ± 427	956 ± 111	241 ± 175
B. merceti (2009)	46% (13)	10	5 (50%)	3 (30%)	2 (20%)	795 ± 882	1372 ± 202	120 ± 67
B. merceti (2010)	52% (21)	21	10 (48%)	5 (24%)	6 (28%)	220 ± 169	329 ± 47	62 ± 41
B. sinuata (2010)	29% (28)	9	5 (56%)	3 (33%)	1 (11%)	393 ± 338	661 ± 141	118 ± 104
B. zonata (2010)	26% (23)	7	4 (57%)	2 (29%)	1 (14%)	409 ± 389	796 ± 129	231 ± 198

Nesting patches largely overlapped between species (Fig. 9), but the nests of each species were not particularly close to heterospecific nests, suggesting that females are neither repulsed by, nor attracted to, congenerics. Thus, the spatial arrangement of the nests of *Bembix* digger wasps seems to be primarily the result of female-female attraction during nest-settlement decisions, in accordance with both the "tradition" and the "copying" mechanisms suggested for nesting vertebrates.

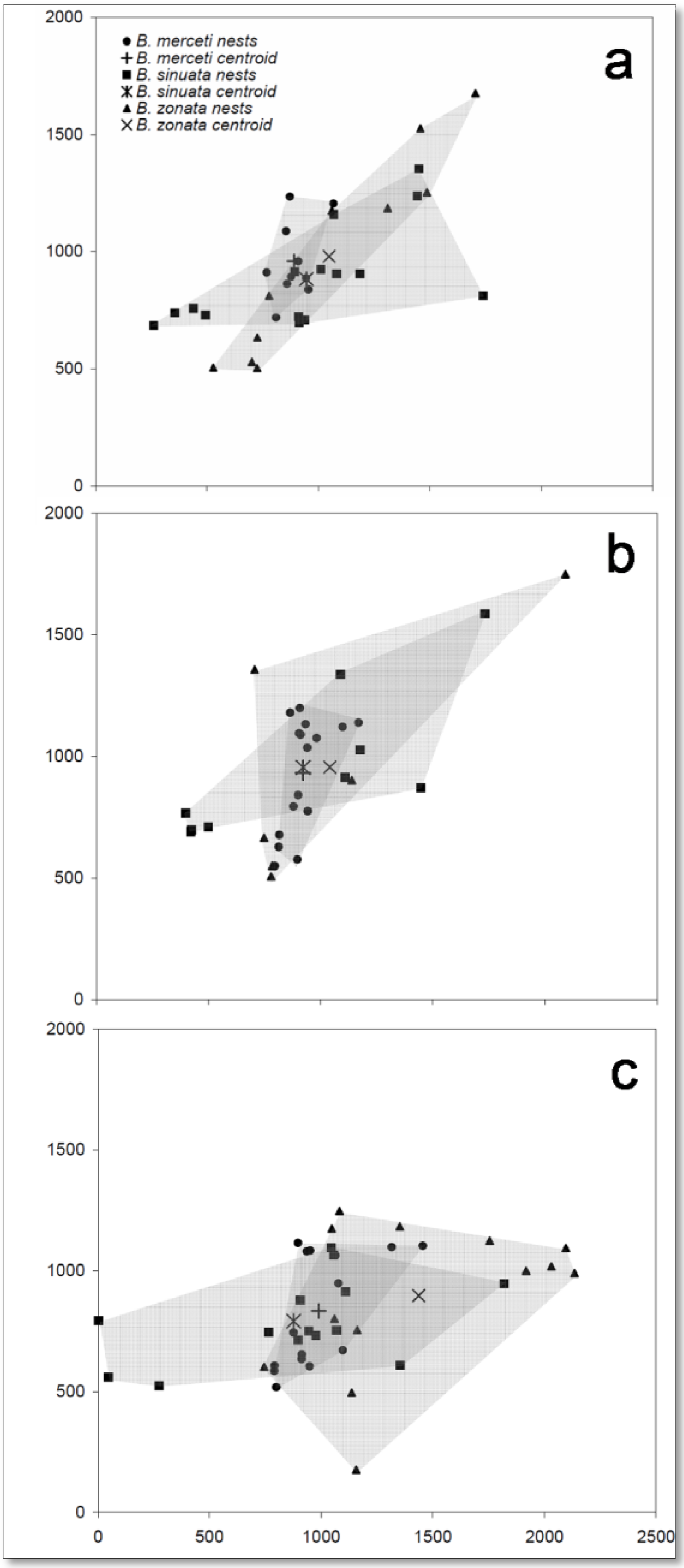

Figure 9. Minimum convex polygons including all the nests recorded for the three *Bembix* species, in the three different periods of the nesting season in 2010 (a-c, 1st, 2nd and 3rd period respectively).

CHAPTER 6

Chemical communication between the cuckoo wasp *Parnopes grandior* (Hymenoptera: Chrysididae) and its hosts, digger wasps of the genus *Bembix* (Hymenoptera: Crabronidae)

Wasp species that store great amounts of provisions into their nests are particularly interesting targets for certain parasites (e.g. cleptoparasites or cuckoo wasps). The interests of parasites and hosts are highly diverging, as the former ones must enhance their strategies to avoid detection by hosts, whereas the seconds should improve their mechanisms to detect and avoid parasites. Cuckoo wasps may remain unnoticed inside their hosts' nests by means of an odour which is similar to their hosts' one (mimicry or camouflage) or may alternatively possess a weak odour (chemical insignificance).

In the present investigation, specimens belonging to three *Bembix* digger wasps, as well as to the chrysidid *Parnopes grandior*, were chemically analysed to describe the types and amount of cuticular hydrocarbons (CHCs) present in them, as well as to find out whether strategies related to mimicry, camouflage or chemical insignificance are being carried out by the parasite. Chemical analyses were supported by the development of behavioural tests (*circle tube* tests, where the kind of response of the females of the different wasp species towards the presence of *P. grandior* and of a control chrysidid, *Hedychrum longicolle*, which parasitizes a different host (bees), was recorded), as well as by the obtaining of cocoons to assess the degree of parasitism exerted by *P. grandior*. If mimicry, camouflage or chemical insignificance were taking place in the studied *Bembix-P.grandior* systems, it would be expectable the host species not to detect *P.*

grandior in the *circle tube* tests, but to recognize *Hedychrum longicolle*. Additionally, the CHC profiles of *P. grandior* and of the different *Bembix* species should be similar (if either mimicry or camouflage exists), or the amount of CHCs should be lower in *P. grandior* (in the case that insignificance is taking place).

Bembix species were neither significantly more aggressive nor more tolerant towards *P. grandior* than towards *H. longicolle*. Nevertheless, *B. merceti* was the only species never being aggressive against the chrysidids, and the one that most often avoided interactions with them (Table 7).

Table 7. Mean number of events per minute (± SD) of each kind of behaviour (AGG: aggression; TOL: tolerance/cooperation; AV: avoidance), for the six types of *circle tube* tests performed. In brackets, the number of tests done for each type is expressed.

	Mean number of events per minute		
Type of *circle tube* test (N)	AGG	TOL	AV
B. merceti vs. P. grandior (12)	0	0.342 ± 0.439	1.193 ± 1.104
B. merceti vs. H. longicolle (8)	0	0.558 ± 0.784	1.183 ± 1.254
B. sinuata vs. P. grandior (8)	0.267 ± 0.479	0.293 ± 0.306	0.737 ± 0.872
B. sinuata vs. H. longicolle (7)	0.402 ± 0.447	0.528 ± 0.893	0.124 ± 0.227
B. zonata vs. P. grandior (12)	0.247 ± 0.411	0.477 ± 0.680	0.222 ± 0.324
B. zonata vs. H. longicolle (13)	0.245 ± 0.304	0.302 ± 0.328	0.374 ± 0.330

The ANOSIM revealed that *P. grandior* is chemically much more similar to *B. merceti* than the remainder of the *Bembix* (Fig. 10), suggesting the existence of chemical mimicry between both species.

On the other hand, evidence of chemical insignificance (*sensu stricto*) by *P. grandior* was not detected, although the presence of great amounts of long-chain CHCs in *P. grandior* could make difficult the detection of the parasite by *B. zonata* and *B. sinuata*, enabling the existence of a certain degree of parasitism.

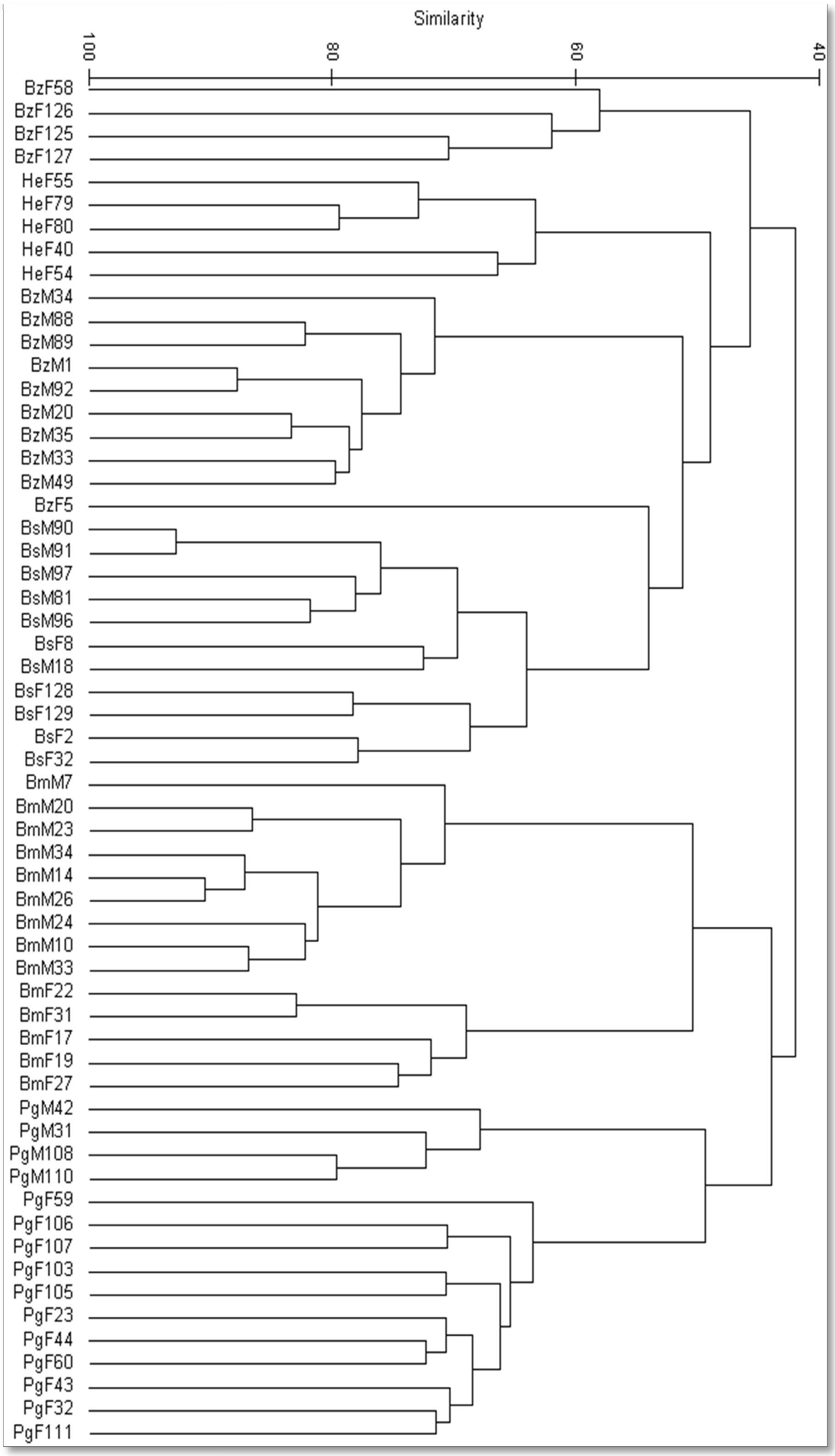

Figure 10. *Cluster* showing the degree of CHC proximity of the individuals of the different species and sexes studied. Note that *P. grandior* and *B. merceti* are more similar between them than *B. merceti* and the rest of the species of its own genus. PgF: *P. grandior* females; PgM: *P. grandior* males; BmF: *B. merceti* females; BmM: *B. merceti* males; BsF: *B. sinuata* females; BsM: *B. sinuata* males; BzM: *B. zonata* males; HeF: *H. longicolle* females; BzF: *B. zonata* females.

CONCLUSIONS

First.- *Bembix merceti* is selective with respect to both the taxa and size of its captures. However, taxonomic selectivity seems to depend on the tendency to prey preferentially upon large dipterans (even though the taxa they belong to are less abundant in the environment); additionally, the females select the largest individuals within those fly families which have not a particularly high mean mass, probably to optimize its provisioning flights.

Second.- Although *B. merceti* captures prey in accordance with the general predictions of the *ODT* for *central-place foragers*, the females maintain a margin with respect to their maximum theoretical load capacity, in such a way that it can be considered that the species carries out a suboptimal foraging. The scarcity of big prey in the environment, the type of provisioning in the species (progressive), the great manoeuvrability of their prey or the pressure exerted by their natural enemies could be some of the factors accounting for this fact. The margin with the maximum load capacity is even wider in *Bembix zonata* (a species which would also forage in a suboptimal way); this could constitute a strategy by the females to reduce the duration of their provisioning flights, due to the greater parasitic pressure that the chrysidid *Parnopes grandior* seems to exert on this wasp species.

Third.- Several biometric traits related to prey flight capacity seem to have an influence on the choice of prey by the different wasp species: a certain degree of segregation is evident between the prey of *B. merceti* and those of *B. zonata*, in such a way that the first one hunts for prey with greater *WL*, but lower *FMR*, than the second one. The *FMR* of the females of *B. merceti* and *B. zonata* is species-specific, and higher in *B. zonata*, which consequently has a greater capacity for load transportation in flight. However, *WL* increases with wasp mass, regardless of the species it belongs to, probably owing to the static size of the wings.

Fourth.- The populations of *B. merceti*, *B. zonata* and *Stizus continuus* exhibit, in every generation or year, inter-individual variation of prey species, while only in some populations/years there is variation of prey size among females.

Fifth.- Morphological distance between females and the distance between their nests are factors explaining taxonomic and/or size prey dissimilarity only in some of the populations/years analysed. Although such factors may be rarely important in wasp resource partitioning, the fact that in the most aggregated population (*B. zonata* in 2009) females nesting in close vicinity had a greater taxonomic prey overlap, suggests that aggregation patterns in certain conditions (e.g. high density) may be important in the partitioning of resources at an inter-individual level.

Sixth.- There is a generalized inter-individual network-based variation through the different flying seasons in *B. merceti*, both for prey taxa and sizes, probably owing to the acquisition of specific hunting strategies by different females. The population showed nestedness in two of the flying seasons (2008 and 2010), due to the simultaneous existence of generalist (of a bigger size) and specialist (of a smaller size) females, as well as to the capture of the most abundant species by a higher number of females, whereas in a third flying season (2009) the population was organized in clusters.

Seventh.- *Bembix merceti*, *B. zonata* and *B. sinuata* establish their nests in groups in the early moments of the flying season, while later in the season their distribution generally exhibits a trend towards random patterns, for distances over 350 cm. Female-female attraction within species accounts significantly for clumped patterns of nests, while the position of the previous nest plays a role of limited importance. The great influence of the female-female attraction on the constitution of aggregations could be due to the existence of a “copy behaviour”, in which the females would use places where other nests have been successfully dug and provisioned as a cue for the establishment of their own nests.

Eighth.- Although *B. merceti*, *B. zonata* and *B. sinuata* greatly overlap their nesting areas, the females don’t nest particularly near their congenerics’ nests.

Ninth.- The different species and sexes studied can be properly defined on the basis of the total CHCs, or only of the alkenes, but not on the basis of only the alkanes; thus, the alkenes could play a more important role in the recognition of natural enemies.

Tenth.- *Bembix merceti* and *P. grandior* possess a high degree of similarity of their CHCs, probably owing to the existence of chemical mimicry in *P. grandior* with respect to *B. merceti*. Although the existence of a strategy of chemical insignificance (*sensu stricto*) can be discarded in *P. grandior*, in the systems *B. zonata*-*P. grandior* and *B. sinuata*-*P. grandior* a particular kind of chemical insignificance could be acting, due to the presence of a main proportion of long-chain CHCs in the parasitoid, which could favour the existence of a certain level of parasitism, observed in both host species.

REFERENCES

Abeille, E. (1878): *Diagnoses de Chrysides nouvelles*. Société Entomologique de France, Marseille.

Alcock, J. (1975a): The behavior of western cicada-killer males, *Sphecius grandis* (Sphecidae, Hymenoptera). *J. Nat. Hist*. **9**: 561 - 566.

Alcock, J. (1975b): The nesting behavior of *Philanthus multimaculatus* Cameron (Hymenoptera: Sphecidae). *Amer. Midl. Nat*. **93**: 222 - 226.

Alcock, J. (1975c): Territorial behaviour by males of *Philanthus multimaculatus* (Hymenoptera: Sphecidae) with a review of territoriality in male sphecids. *Anim. Behav.* **23**: 889 - 895.

Alcock, J. (2001): The natural history of a miltogrammine fly, *Miltogramma rectangularis* (Diptera: Sarcophagidae). *J. Kansas Entomol. Soc.* **73**: 208 - 219.

Alexander, B. A. (1985): Predator–prey interactions between the digger wasp *Clypeadon laticinctus* and the harvester ant *Pogonomyrmex occidentalis*. *J. Nat. Hist.* **19**: 1139 - 1154.

Alexander, B. A. and Asís, J. D. (1997): Patterns of nest occupancy and provisioning in *Cerceris rufopicta* Smith (Hymenoptera: Sphecidae). *J. Ins. Behav.* **10**: 871 - 893.

Almbro, M. and Kullberg, C. (2008): Impaired escape flight ability in butterflies due to low flight muscle ratio prior to hibernation. *J. Exp. Biol.* **211**: 24 - 28.

Almbro, M. and Kullberg, C. (2009): The downfall of mating - the effect of mate-carrying and flight muscle ratio on the escape ability of a pierid butterfly. *Behav. Ecol. Sociobiol*. **63**: 413 - 420.

Almeida-Neto, M., Guimarães, P., Guimarães, P. R., Loyola, R. D. and Ulrich, W. (2008): A consistent metric for nestedness analysis in ecological systems: reconciling concept and measurement. *Oikos* **117**: 1227 - 1239.

Almeida-Neto, M. and Ulrich, W. (2011): A straightforward computational approach for measuring nestedness using quantitative matrices. *Environm. Modell. Software* **26**: 173- 178.

Araújo, M. S., Bolnick, D. I. and Layman, C. A. (2011): The ecological causes of individual specialisation. *Ecol. Lett.* **14**: 948 - 958.

Araújo, M. S. and Gonzaga, Marcelo O. (2007): Individual specialization in the hunting wasp *Trypoxylon* (*Trypargilum*) *albonigrum* (Hymenoptera, Crabronidae). *Behav. Ecol. Sociobiol.* **61**: 1855 - 1863.

Araújo, M. S., Guimarães, P. R., Svanbäck, R., Pinheiro, A., Guimarães,P., dos Reis, S. F. and Bolnick, D. I. (2008): Network analysis reveals contrasting effects of intraspecific competition on individual vs. population diets. *Ecology* **89**: 1981 - 1993.

Araújo, M. S., Martins, E. G., Cruz, L. D., Fernandes, F. R., Linhares, A. X., dos Reis S. R., and Guimarães, P. R. (2010): Nested diets: a novel pattern of individual-level resource use. *Oikos* **119**: 81 - 88.

Asís, J. D., Gayubo, S. F. and Tormos, J. (1992): Data on the nesting behaviour of five European *Bembix* and description of the mature larvae of *B. merceti* and *B. rostrata* (Hymenoptera, Sphecidae). *Deut. Entomol. Zschr.* **39**: 221 - 231.

Asís, J. D., Tormos, J. and Gayubo, S. F. (1996): Behavior of *Philanthus pulchellus* (Hymenoptera: Sphecidae) with a description of its mature larva. *Ann. Entomol. Soc. Amer.* **89:** 452 - 458.

Asís, J. D., Tormos, J. and Gayubo, S. F. (2004): Nesting behaviour and provisioning in *Bembix merceti* and *Bembix zonata* (Hymenoptera: Crabronidae). *J. Nat. Hist.* **38**: 1799 - 1809.

Asís, J. D., Tormos, J. and Gayubo, S. F. (2006): Territorial dynamics and contest behaviour in the solitary wasp *Stizus continuus* (Hymenoptera: Apoidea: Crabronidae). *Behaviour* **143**: 83 - 104.

Asís, J. D., Tormos, J. and Jiménez, R. (1988): Contribution to the study of the biology of *Stizus continuus* (Hymenoptera: Sphecidae): *Entomol. News* **99**: 199 - 206.

Baerends, G. P. (1941): Fortppflanzungsverhalten und Orientierung der Grabwespe *Ammophila campestris Jur. Tijdschr. Entomol.* **84**: 68 - 275.

Bagnères, A. G. and Lorenzi, M. C. (2010): Chemical deception/ mimicry using cuticular hydrocarbons. In: *Insect Hydrocarbons. Biology, Biochemistry, and Chemical Ecology* (Blomquist, G. J. and Bagnères, A. G., eds.). Cambridge University press, UK, p. 282 - 324.

Ballesteros, Y., Tormos, J., Gayubo, S. F. and Asís, J. D. (2012): Notes on the prey, nesting behaviour and natural enemies of three *Bembix* sand wasps (Hymenoptera: Crabronidae) in the Iberian Peninsula. *Ann. Soc. Entomol. Fr.* **48**: 281 - 288.

Batagelj, V. and Mrvar, A. (1998): Pajek-Program for Large Network Analysis. *Connections* **21**: 47 - 57.

Bernard, F. (1934): Observations sur les proies de quelques hyménoptères. *Bull. Soc. Entomol. Fr.* **39**: 247-250.

Bernard, F. (1935): Hyménoptêres prédateurs des environs de Fréjus. *Ann. Soc. Entomol. Fr.* **104**: 31 - 72.

Blomquist, G. J. (2010) : Structure and analysis of insect hydrocarbons. In : *Insect Hydrocarbons. Biology, Biochemistry, and Chemical Ecology* (Blomquist, G. J. and Bagnères, A. G., eds.). Cambridge University press, UK, pp. 19 - 34.

Blomquist, G. J and Bagnères, A. G. (2010): Introduction: history and overview of insect hydrocarbons. In: *Insect Hydrocarbons. Biology, Biochemistry, and Chemical Ecology* (Blomquist, G. J. and Bagnères, A. G., eds.). Cambridge University press, UK, pp. 3 - 18.

Boesi, R., Polidori, C., **Gayubo, S., Tormos, J., Asís J. D. and Andrietti F.** (2007): Nesting biology, morphological remarks and description of the mature larva of *Mellinus arvensis obscurus* (Hymenoptera: Crabronidae) in Nepal. *Flo. Entomol.* **90**: 184 - 190.

Boesi, R., Polidori C. and Andrietti F. (2009): Biology of *Lasioglossum (L.) majus* (Hymenoptera: Halictidae), a largely solitary sweat bee with behavioural adaptations to communality. *J. Ethol.* **27**: 361 - 367.

Bohart, R. M. and Menke, A. S. (1976): *Sphecid wasps o the World: a generic revision.* University of California Press, Berkeley.

Bolnick, D. I. and Paull, J. E. (2009): Morphological and dietary differences between individuals are weakly but positively correlated within a population of threespine stickleback. *Evol. Ecol. Res.* **11**: 1217 - 1233.

Bolnick, D. I., Svanbäck, R., Araújo, M. S. and Persson, L. (2007): Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Ecology* **104**: 10075 - 10079.

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D. and Forister, M. L. (2003): The ecology of individuals: incidence and implications of individual specialization. *Am. Nat.* **161**: 1 – 28.

Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. M. and Svanbäck, R. (2002): Measuring individual-level resource specialization. *Ecology* **83**: 2936 - 2941.

Brandt, M., Heinze, J., Schmitt, T. and Foitzik, S. (2005): A chemical level in the coevolutionary arms race between an ant social parasite and its hosts*. J. Evol. Biol.* **18**: 576 - 586.

Brockmann, H. J. (1979): Nest-site selection in the great golden digger wasp, *Sphex ichneumoneus* L. (Sphecidae). *Ecol. Entomol.* **4**: 211 - 224.

Brown, C. R. and Brown, M. B. (1986): Ectoparasitism as a cost of coloniality in cliff swallows (*Hirundo pyrrhonota*). *Ecology* **67**: 1206 – 1218.

Buckner, J. L. (1993): Cuticular polar lipids in insects. In: *Insect Lipids: Chemistry, Biochemistry and Biology* (Stanley-Samuelson, D.W. and Nelson, D.R., eds.). University of Nebraska Press, Lincoln, Nebraska, pp. 227 - 270.

Casiraghi, M., Polidori, C., Ferreri, P., Preatoni, D. G., Andrietti, F. and Martinoli, A. (2003): Does the distance among nest clusters affect reproductive success in *Ammophila sabulosa* (Hymenoptera, Sphecidae)? *Ethol. Ecol. Evol.***15**: 329 - 341.

Chai, P. and Srygley, R. B. (1990): Predation and the flight, morphology, and temperature of neotropical rain-forest butterflies. *Am. Nat*. **135**: 398- 411.

Clausen, C. P. (1940): *Entomophagous insects*. McGraw-Hill, NY.

Coelho, J. R. (1997): Sexual size dimorphism and flight behavior in cicada killers (*Sphecius speciosus*). *Oikos* **79**: 371 - 375.

Coelho, J. R. (2011): Effects of prey size and load carriage on the evolution of foraging strategies in wasps. In: *Predation in the Hymenoptera: an evolutionary perspective* (Polidori, C., ed.). Transworld Research Network, Kerala, India, pp. 23 – 38.

Coelho, J. R., Hastings, J., Holliday, C. W. and Mendell, A. (2008): Load carriage during foraging in two species of solitary wasps. *J. Hym. Res.* **17**: 57 - 63.

Coelho, J. R. and Ladage, L. D. (1999): Foraging capacity of the great golden digger wasp *Sphex ichneumoneus. Ecol. Entomol.* **24**: 480 - 483.

Colwell, R. K. and Futuyma, D. J. (1971): On the measurement of niche breadth and overlap. *Ecology* **52**: 567 - 576.

Danks, H. V. (1970): Biology of some stem-nesting aculeate Hymenoptera. *Trans. R. Entomol. Soco. Lond.* **122**: 323 - 399.

Davies, N. B., Bourke, A. F. G. and Brooke, M. D. (1989): Cuckoos and parasitic ants - interspecific brood parasitism as an evolutionary armsrace. *Trends Ecol. Evol.* **4**: 274 - 278.

Dawkins, R. and Krebs, J. R. (1979): Arms races between and within species. *P. Roy. Soc. Lond. B. Bio.* **205**: 489 - 511.

Dodson, G. N. and Yeates, D. K. (1989): Male *Bembix furcata* Erichson (Hymenoptera: Sphecidae) behaviour on a hilltop in Queensland. *Pan-Pac. Entomol.* **65**: 172 - 175.

Domenici, P. (2001): The scaling of locomotor performance in predator-prey encounters: from fish to killer whales. *Comp. Biochem. Phys. A* **131**: 169 - 182.

Duncan, R. P. (1991): Competition and the coexistence of species in a mixed podocarp stand. *J. Ecol.* **69**: 559 - 564.

Emlen, J. M. (1966): The role of time and energy in food preference. *Am. Nat.* **100**: 611 - 617.

Enslin, E. (1929): Beiträge zur Metamorphose del Goldwespen. *Zeitschr. Wissens. Insektenbiol.* **24**: 116 - 130.

Evans, H. E. (1955): An ethological study of the digger wasp *Bembecinus neglectus*, with a review of the ethology of the genus. *Behaviour* **7**: 287 - 303.

Evans, H. E. (1957): *Studies on the comparative ethology of digger wasps of the genus* Bembix. Comstock Publishing Associates. Ithaca, NY.

Evans, H. E. (1959): Observations on the nesting behaviour of digger wasps of the genus *Ammophila*. *Amer. Midl. Nat.* **62**: 449 - 473.

Evans, H. E. (1966a): *The comparative ethology and evolution of the sand wasps*. Harvard University Press, Cambridge, MA.

Evans, H. E. (1966b): The accessory burrows of digger wasps. *Science* **152**: 465 - 471.

Evans, H. E. and Hook, A. W. (1986): Nesting behaviour of *Cerceris* digger wasps, with special reference to nest reutilization and nest sharing (Hymenoptera: Sphecidae). *Sociobiology* **11**: 275 - 302.

Evans, H. E. and Matthews, R. W. (1973): *Systematics and nesting behavior of Australian* Bembix *sand wasps (Hymenoptera, Sphecidae).* The American Entomological Institute, Michigan, U. S. A.

Evans, H. E. and O'Neill, K. M. (1988): *The natural history and behavior of North American beewolves*. Cornell University Press, Ithaca, NY.

Evans, H. E. and O’Neill, K. M. (2007). *The sand wasps. Natural history and behavior*. Harvard University Press, Cambridge, MA.

Fabre, J. H. (1879): *Souvenirs entomologiques (1ére ser) (nouvelle edition illustrée).* Sciences Nat., Compiègne (1985).

Ferton, C. (1894): Sur les moeurs du *Dolichurus haemorrhous* Costa. *Act. Soc. Linn. Bordeaux* **47**: 215 - 221.

Ferton, C. (1895): Moeurs de *Dinetus pictus* Fabr. (Hyménoptêres). *Bull. Soc. Entomol. Fr.* **3**: 37.

Ferton, C. (1899): Observations sur l’instinct des *Bembex* Fabr. (Hyménoptères). *Act. Soc. Linn. Bordeaux* **54**: 331 - 345.

Ferton, C. (1901): Notes detaches sur l’instinct des hyménoptères mellifères et ravisseurs avec la description de quelques espèces. 1ére ser. *Ann. Soc. Entomol. Fr.* **70**: 83 - 148.

Ferton, C. (1902): Notes detaches sur l’instinct des hyménoptères mellifères et ravisseurs. 2éme ser. *Ann. Soc. Entomol. Fr.* **71**: 499 - 530.

Ferton, C. (1905): Notes sur l’instinct des hyménoptères mellifères et ravisseurs. 3éme ser. *Ann. Soc. Entomol. Fr.* **74**: 56 - 101.

Ferton, C. (1908): Notes sur l’instinct des hyménoptères mellifères et ravisseurs. 4éme ser. *Ann. Soc. Entomol. Fr.* **77**: 535 - 584.

Ferton, C. (1909): Notes detaches sur l’instinct des hyménoptères mellifères et ravisseurs. 5éme ser. *Ann. Soc. Entomol. Fr.* **78**: 401 - 422.

Ferton, C. (1910): Notes detaches sur l’instinct des hyménoptères mellifères et ravisseurs. 6 éme ser. *Ann. Soc. Entomol. Fr.* **79**: 145 - 178.

Ferton, C. (1911): Notes detaches sur l’instinct des hyménoptères mellifères et ravisseurs. 7éme ser. *Ann. Soc. Entomol. Fr.* **80**: 351 - 412.

Ferton, C. (1912): Hyménoptères nouveaux d'Algérie et observations sur l'instinct d'une espèce. *Bull. Soc. Entomol. Fr.* (1912): 186 - 191.

Ferton, C. (1914): Notes detaches sur l'instinct des hyménoptères mellifères et ravisseurs. 8éme ser. *Ann. Soc. Entomol. Fr.* **83**: 81 - 119.

Ferton, C. (1920): Notes detaches sur l'instinct des hyménoptères mellifères et ravisseurs. 9éme ser. *Ann. Soc. Entomol. Fr.* **89**: 329 - 375.

Field, J. (1989): Intraspecific parasitism and nesting success in the solitary wasp *Ammophila sabulosa*. *Behaviour* **110**: 23 - 46.

Field, J. (1992): Patterns of nest provisioning and parental investment in the solitary digger wasp *Ammophila sabulosa*. *Ecol. Entomol* **17**: 43 - 51.

Foitzik, S., Fischer, B. y Heinze, J. (2003): Arms races between social parasites and their hosts: geographic patterns of manipulation and resistance. *Behav. Ecol.* **14**:80 - 88.

Gamboa, G. J. (2004): Kin recognition in eusocial wasps. *Ann. Zool. Fennici* **41**: 789 - 808.

Gauld, I. and Bolton, B. (1988): *The Hymenoptera*. British Museum (Natural History) and Oxford University Press, London and Oxford.

Genise, J.F. (1982): Estudios sobre el comportamiento de Bembicini neotropicales I. *Bicyrtes simillima* (Smith) y *Bicyrtes discisa* (Tasch.) (Hymenoptera: Sphecidae), con una revisión de los tipos de aprovisionamiento en Bembicini. *Physis (Buenos Aires) (C)* **43**: 93 - 99.

Gibbs, A. (1998): Water-proofing properties of cuticular lipids. *Amer. Zool.* **38**, 471 - 482.

Godfray, H. C. J. (1994): *Parasitoids: Behavioral and evolutionary ecology*. Princeton University Press, Princeton, NY.

Grandi, G. (1930): Contributi alla conoscenza biológica e morfológica degli imenotteri melliferi e predatori. XI. *Bol. Lab. Entomol. R. Ist. Sup. Agr. Bologna* **3**: 302 - 343.

Grandi, G. (1934): Contributi alla conoscenza degli imenotteri melliferi e predatori. XI. *Bol. Lab. Entomol. R. Ist. Sup. Agr. Bologna* **7**: 1 - 144.

Grandi, G. (1961): Studi di un entomologo sugli imenotteri superiori. *Boll. Inst. Entomol. Univ. Bologna* **25**: 1 - 659.

Grant, P. R. (2006): Opportunistic predation and offspring sex ratios of cicada-killer wasp (*Sphecius speciosus* Drury). *Ecol. Entomol*. **31**: 539 - 547.

Greenberg, G. (1979): Genetic component of bee odor in kin recognition. *Science* **206**: 1095 - 1097.

Guimarães, P. R. and Guimarães, P. (2006): Improving the analyses of nestedness for large sets of matrices. *Environm. Modell. Software* **21**: 1512 - 1513.

Gwynne, D. T. and Dodson, G. N. (1983): Nonrandom provisioning by the digger wasp, *Palmodes laeviventris* (Hymenoptera: Sphecidae). *Ann. Entomol. Soc. Am.***76**: 434 - 436.

Haase, P. (1995): Spatial pattern analysis in ecology based on Ripley's K-function: introduction and methods of edge correction. *J. Veg. Sci.* **6**: 1651 - 1661.

Hadley, N. F. (1981): Cuticular lipids of terrestrial plants and arthropods: a comparison of their structure composition and waterproofing function. *Biol. Rev.* **56**: 23 - 47.

Hamilton, W. D. (1971): Geometry for the selfish herd. *J. Theor. Biol.* **31**: 195 - 311.

Hastings, J. (1986): Provisioning by female western cicada-killer wasps, *Sphecius speciosus* (Hymenoptera: Sphecidae): influence of body size and emergence time on individual provisioning success. *J. Kans. Entomol. Soc.* **59**: 262 - 268.

Hedenström, A. and Rosén (2001): Predator versus prey: on aerial hunting and escape strategies in birds. *Behav. Ecol.* **12**: 150 - 156.

Hefetz, A., Eickwort, G. C., Blum, M. S., Cane, J. and Bohart, G. E. (1982): A comparative-study of the exocrine products of cleptoparasitic bees (Holcopasites) and their hosts (*Calliopsis*) (Hymenoptera, Anthophoridae, Andrenidae). *J.Chem. Ecol***.** **8**: 1389 - 1397.

Herzner, G., Schmitt, T., Linsenmair, K. E. and Strohm, E. (2005): Prey recognition by females of the European Beewolf and its potential for a sensory trap. *Anim. Behav.* **70**: 1411 - 1418.

Howard, R.W. and Blomquist, G. J. (2005): Ecological, behavioral and biochemical aspects of insect hydrocarbons. *Annu. Rev. Entomol.* **50**: 371 - 393.

Howard, R. W., McDaniel, C. A. and Blomquist, G. J. (1982): Chemical mimicry as an integrating mechanism for three termitophiles associated with *Reticulitermes virginicus* (Banks). *Psyche* **89**: 157 - 167.

Ingram, T., Stutz, W. E. and Bolnick, D.I. (2011): Does intraspecific size variation in a predator affect its diet diversity and top-down control of prey? *PLoS ONE* **6**:e20782.

Jackson, B. D. and Morgan, E. D. (1993): Insect chemical communication: pheromones and exocrines glands of ants. *Chemoecology* **4**:125 - 144.

Jacob-Remacle, A. (1986): Mortalité de quelques hyménoptéres aculéates nidifiant dans des nichoirs-pièges. *Bull. Ann. Soc. R. Belge Entomol.* **122**:107 - 118.

Kendall, B.E. and Fox, G.A. (2002): Unstructured individual variation and demographic stochasticity. *Conserv. Biol.* **16**: 109 - 116.

Kimsey, L. S. and Bohart, R. M. (1990): *The chrysidid wasps of the World*. Oxford University Press, NY.

Kukuk, P. F. and Decelles, P. C. (1986): Behavioural evidence for population structure in *Lasioglossum* (*Dialictus*) *zephyrum* female dispersion patterns. *Behav. Ecol. Sociobiol.* **19**: 233 - 239.

Lahav, S., Soroker, V., Hefetz, A. and Vander Meer, R.K. (1999): Direct behavioural evidence for hydrocarbons as ant recognition discriminators. *Naturwissenschaften* **86**: 246 - 249.

Lankau, R.A. (2009): Genetic variation promotes long-term coexistence of *Brassica nigra* and its competitors. *Am. Nat.* **174**: 40 - 53.

Larsen, O., Gleffe, G. and Tengö, J. (1986): Vibration and sound communication in solitary bees and wasps. *Physiol. Entomol.* **62**: 258 - 268.

Larsson, F. K. (1986): Increased nest density of the digger wasp *Bembix rostrata* as a response to parasites and predators (Hymenoptera: Sphecidae). *Entomol. Gen.* **12**: 71 - 75.

Larsson, F. K. and Larsson, K. (1989): Body size and nesting behaviour of the sand wasp *Stictia heros* (Hymenoptera: Sphecidae) in Costa Rica. *J. Trop. Ecol.* **5**: 117 - 120.

Lichstein, J.W., Dushoff, J., Levin, S. A. and Pacala, S. W. (2007): Intraspecific variation and species coexistence. *Am. Nat.* **170**: 807 - 818.

Linsley, E. G. and MacSwain, J. W. (1956): Some observations on the nesting habits and prey of *Cerceris californica* Cresson (Hymenoptera: Sphecidae). *Ann. Entomol. Soc. Amer.* **49**: 71 - 84.

Lockey, K. H. (1988): Lipids of the insect cuticle: origin, composition and function. *Comp. Biochem. Physiol. B- Biochem. Mol. Biol.* **89**: 595 - 645.

Lomnicki, A. (1988): *Population Ecology of Individuals*. Princeton University Press, Princeton, NJ.

Lucas, C., Pho, D. B., Jallon, J. M. and Fresneau, D. (2005): Role of cuticular hydrocarbons in the chemical recognition between ant species in the *Pachycondyla villosa* species complex. *J. Insect Physiol.* **51**: 1148 - 1157.

Lüps, P. (1969): Die Grabwespe *Bembecinus tridens* (Fabricius) (Hymenoptera, Sphecidae). *Jahr. Naturhist. Mus. St Bern* **3**: 201 - 215.

Lyon, B. E. (2004): An obligate brood parasite trapped in the intraspecific arms race of its hosts. *Nature* **432**: 390 - 393.

MacArthur, R. H. and Pianka, E. R. (1966): On optimal use of a patchy environment. *Am. Nat.* **100**: 603 - 609.

Marden, J. H. (1987): Maximum lift production during takeoff in flying animals. *J. Exp. Biol.* **130**: 235 - 258.

Martin, S. J., Carruthers, J. M., Williams, P. H. and Drijfhout, F. P. (2010): Host specific social parasites (*Psithyrus*) indicate chemical recognition system in bumblebees. *J. Chem. Ecol.* **36 (8)**: 855 - 863.

McCorquodale, D. B. (1985): Digger wasps (Hymenoptera: Sphecidae) provisioning flights as a defence against a nest parasite, *Senotainia trilineata* (Diptera: Sarcophagidae). *Can. J. Zool.* **64:** 1620 - 1627.

Michener, C. D., Lange, R. B., Bigarella, J. J. and Salamuni, R. (1958): Factors influencing the distribution of bees in earth banks. *Ecology* **39**: 207 - 217.

Mingo, E. (1994): Hymenoptera, Chrysididae. In: *Fauna Ibérica, vol 6*. (Ramos, M. A. *et al*., eds.). CSIC, Museo Nacional de Ciencias Naturales, Madrid.

Newcomer, E. J. (1930): Notes on the habits of a digger wasp and its inquiline flies. *Ann. Entomol. Soc. Amer* **23**: 552 - 563.

Norberg, U. M. and Rayner, J. M. V. (1987): Ecological morphology and flight in bats (Mammalia: Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Phil. Trans. R. Soc. Lond. G.* **316**: 335 - 427.

Nordell, S. E. and Valone, T. J. (1998): Mate choice copying as public information. *Ecol. Lett.* **1**: 74 - 76.

Okuyama, T. (2008): Individual behavioral variation in predator-prey models. *Ecol. Res.* **23**: 665 - 671.

O'Neill, K. M. (1983): The significance of body size in territorial interactions of male bee-wolves (Hymenoptera: Sphecidae, *Philanthus*). *Anim. Behav.* **31**: 411 - 414.

O'Neill, K. M. (2001): *Solitary Wasps: Behaviour and Natural History*. Cornell University Press, Ithaca, NY.

O'Neill, K. M. and Evans, H. E. (1983): Body size and alternative mating tactics in the beewolf *Philanthus zebratus* (Hymenoptera: Sphecidae). *Biol. J. Linn. Soc*. **20**: 175 - 184.

Pielou, E. C. (1972): Niche width and niche overlap: a method for measuring them. *Ecology* **53**: 687 – 692.

Pires, M. M., Guimarães, P. R., Araújo, M. S., Giaretta, A. A., Costa, J. C. L. and dos Reis, S. F. (2011): The nested assembly ofindividual-resource networks. *J. Anim. Ecol.* **80**: 896 – 903.

Polidori C., Bevacqua, S. and Andrietti, F. (2010b): Do digger wasps time their provisioning activity to avoid cuckoo wasps (Hymenoptera: Crabronidae and Chrysididae)? *Acta Ethol*. **13**: 11 - 21.

Polidori, C., Boesi, R., Isola, F. and Andrietti, F. (2005): Provisioning patterns and choice of prey in the digger wasp *Cerceris arenaria* (Hymenoptera: Crabonidae): the role of prey size. *Eur. J. Entomol.* **102**: 801 - 804.

Polidori, C., Casiraghi, M., di Lorenzo, M., Valarani and B. and Andrietti, F. (2006a): Philopatry, nest choice and aggregation temporal-spatial change in the digger wasp *Cerceris arenaria* (Hymenoptera: Crabronidae). *J. Ethol.* **24**: 155 – 163.

Polidori, C., Federici, M., Papadia, C. and Andrietti, F. (2006b): Nest sharing and provisioning activity of females of the digger wasp, *Cerceris rubida* (Hymenoptera: Crabronidae). *Ital. J. Zool.* **73**: 55 - 65.

Polidori, C., Giordani, I., Mendiola, P., Asís, J. D., Tormos, J. and Selfa, J. (2010a): Emergence and dispersal relative to natal nest in the digger wasp *Stizus continuus* (Hymenoptera: Crabronidae). *C. R. Biol*. **333**: 255-264.

Polidori, C., Gobbi, M., Chatenaud, L., Santoro, D., Montani, O. and Andrietti, F. (2010c): Taxon-biased diet preference in the "generalist" beetle-hunting wasp *Cerceris rubida* provides insights on the evolution of prey specialization in apoid wasps. *Biol. J. Linn. Soc.* **99:** 544 - 558.

Polidori, C., Mendiola, P., Asís, J.D., Tormos, J., García, M.D. and Selfa, J. (2009): Predatory habits of the grasshopper-hunting wasp *Stizus continuus* (Hymenoptera: Crabronidae): diet preference, predator-prey size relationships and foraging capacity. *J. Nat.Hist.* **43**: 2985 - 3000.

Polidori, C., Mendiola, P., Asís, J. D., Tormos, J., Selfa, J. and Andrietti, F. (2008): Female-female attraction influences nest establishment in the digger wasp *Stizus continuus* (Hymenoptera: Crabronidae). *Anim. Behav.* **75**: 1651 – 1661.

Polidori, C., Santoro, D. and Blüthgen, N. (2013): Does prey mobility affect niche width and individual specialization in hunting wasps? A network-based analysis. *Oikos* **122**: 385 - 394.

Potts, S. G. and Willmer, P. (1997): Abiotic and biotic factors influencing nest-site selection by *Halictus rubicundus*, a ground-nesting halictine bee. *Ecol. Entomol.* **22**: 319 - 328.

Potts, S. G. and Willmer, P. (1998): Compact housing in built-up areas: spatial patterning of nests in aggregations of a ground- nesting bee. *Ecol. Entomol.* **23**: 427 - 432.

Pulawski, W. J. (2012): Catalog of the Sphecidae sensu lato (= Apoidea excluding Apidae). California Academy of Sciences. http://research.calacademy.org/ent/catalog_sphecidae.

Pyke, G. H. (1984): Optimal foraging theory: a critical review. *Ann. Rev. Ecol. Syst.* **15**: 523 – 575.

Quicke, D. L. J. (1997): *Parasitic Wasps.* Chapman and Hall, NY.

Quinn, G. P. and Keough, M. J. (2002): *Experimental design and data analysis for biologists.* Cambridge University Press, UK.

Riddick, E. W. (1992): Nest distribution of the solitary bee *Andrena macra* Mitchell (Hymenoptera: Andrenidae), with observations on nest structure. *P. Entomol. Soc. Wash.* **94**: 568 - 575.

Ripley, B. D. (1976): The second order analysis of stationary processes. *J. Appl. Probab.* **13**: 255 - 266.

Ristich, S. S. (1956): The host relationship of a miltogrammid fly *Senotainia trilineata* (VDW). *Ohio J. Sci*. **56**: 271 - 274.

Rosenheim, J. A. (1988): Parasite presence acts as a proximate cue in the nest-site selection process of a solitary digger wasp *Ammophila dysmica* (Hymenoptera: Sphecidae). *J. Ins. Behav. I*: 333 - 342.

Rosenheim, J. A. (1990): Density-dependent parasitism and the evolution of aggregated nesting in the solitary Hymenoptera. *Ann. Entomol. Soc. Amer.* **83**: 277 - 286.

Roughgarden, J. (1974): Niche width: biogeographic patterns among *Anolis* lizard populations. *Am. Nat.* **108**: 429 - 442.

Rubink, W. L. (1982): Spatial patterns in a nesting aggregation of solitary wasps: Evidence for the role of conspecifics in nest-site selection. *J. Kans. Entomol. Soc.* **55**: 52 - 56.

Rudolf, V. H. W. and Rödel, M. O. (2005): Oviposition site selection in a complex and variable environment: the role of habitat quality and conspecific cues. *Oecologia* **142**: 316 - 325.

Ruther, J., Sieben, S. and Schricker, B. (2002): Nestmate recognition in social wasps: manipulation of hydrocarbon profiles induces aggression in the European hornet. *Naturwissenschaften* **89**: 111 - 114.

Santoro, D., Polidori, C., Asís, J. D. and Tormos, J. (2011): Complex interactions between components of individual prey specialization affect mechanisms of niche variation in a grasshopper-hunting wasp. *J. Anim. Ecol.* **80**: 1123 - 1133.

Schöne, H. and Tengö, J. (1981): Competition of males, courtship behaviour and chemical communication in the digger wasp *Bembix rostrata* (Hymenoptera, Sphecidae). *Behaviour* **77**: 44 - 66.

Semenov, A. P. and Nikolskaya, M. N. (1954): Sistema triby Hedychrini Mocs. (Hym. Chrysididae) y opisaniye novij vidov. *T. Zoolog. In. Acad. Nauk SSSR* **15**: 138 - 145.

Sih, A. and Christensen, B. (2001): Optimal diet theory: when does it work, and when and why does it fail? *Anim. Behav.* **61**: 379 - 390.

Singer, T. L. (1998): Roles of Hydrocarbons in the recognition systems of insects. *Amer. Zool.* ***38***: 394 - 405.

Smith, T. B. (1990): Resource use by bill morphs of an African finch: evidence for intraspecific competition. *Ecology* **71**: 1246 - 1257.

Spofford, M. G. and Kurczewski, F. E. (1990): Comparative larvipositional behaviours and cleptoparasitic frequencies of Nearctic species of Miltogrammini (Diptera: Sarcophagidae). *J. Nat. Hist.* **24**: 731 - 755.

Spofford, M. G. and Kurczewski, F. E. (1992): Counter-cleptoparasitic behaviours of species of Sphecidae (Hymenoptera) in response to Miltogrammini larviposition (Diptera: Sarcophagidae). *J. Nat. Hist.* **26**: 993 - 1012.

Stephens, D. W. and Krebs, J. R. (1986): *Foraging theory*. Princeton University Press, Princeton, NJ.

St. Leger, R. J. (1995): Integument as a barrier to microbial infections. In: *Physiology of the Insect Epidermis* (Binnington, K. and Retnakaran, I., eds.). CSIRO Publications, Melbourne, pp. 284 - 306.

Stone, L. and Roberts, A. (1990). The checkerboard score and species distributions. *Oecologia* **85**: 74 - 79.

Strohm, E., Kroiss, J., Herzner, G., Laurien-Kehnen, C., Boland, W., Schreier, P. and Schmitt, T. (2008): A cuckoo in wolves' clothing? Chemical mimicry in a specialized cuckoo wasp of the European beewolf (Hymenoptera, Chrysididae and Crabronidae). *Front. Zool.* **5**: 2.

Strohm, E., Laurien-Kehnen, C. and Bordon, S. (2001): Escape from parasitism: spatial and temporal strategies of a sphecid wasp against a specialised cuckoo wasp. *Oecologia* **129**: 50 - 57.

Strohm, E. and Linsenmair, K. E. (1999): Measurement of parental investment and sex allocation in the European beewolf *Philanthus triangulum* (Hymenoptera: Sphecidae). *Behav. Ecol. Sociobiol.* **47**: 76 - 88.

Strohm, E. and Marliani, A. (2002): The cost of parental care: prey hunting in a digger wasp. *Behav. Ecol.* **13**: 52 - 58.

Svanbäck, R. and Bolnick, D. I. (2005): Intraspecific competition affects the strength of individual specialization: an optimal diet theory method. *Evol. Ecol. Res.* **7**: 993 – 1012.

Svanbäck, R. and Persson, L. (2004): Individual diet specialization, niche width and population dynamics: implications for trophic polymorphisms. *J. Anim. Ecol.* **73**: 973 - 982.

Takahashi, S. and Gassa, A. (1995): Roles of cuticular hydrocarbons in intra- and interspecific recognition behavior of two Rhinotermitidae species. *J. Chem. Ecol.* **21**: 1837 - 1845.

Tengö J. and Bergstrom, G. (1977): Cleptoparasitism and odor mimetism in bees - Do *Nomada* males imitate odor of *Andrena* females? *Science* **196 (4294)**: 1117 - 1119.

Tinbergen, N. (1932): Über die Orientierung des Bienenwolfes (*Philanthus triangulum* Fabr.). *Zeits. Verg. Physiol.* **16**: 305 - 335.

Tinbergen, N. (1935): Über die Orientierung des Bienenwolfes. II. Die Bienenjagd. *Zeits. Verg. Physiol.* **21**: 699 - 716.

Tormos, J., Asís, J. D., Beneitez, A. and Gayubo, S. F. (2009): Description of the mature larva of the sand wasp *Bembix bidentata* and its parasitoids (Hymenoptera: Crabronidae, Chrysididae, Mutillidae). *Fla. Entomol.* **92**: 43 - 53.

Treherne, J. E. and Foster, W. A. (1980): The effects of group size on predator avoidance in a marine insect. *Animal Behaviour* **28**: 1119 - 1122.

van Iersel, J. J. A. (1952): On the orientation of *Bembex rostrata* L. *Trans. Ninth Internat. C. Entomol.* **1**: 384 - 393.

Vogel, S. (2008): Modes and scaling in aquatic locomotion. *Integr. Comp. Biol.***6**: 702 - 712.

Wcislo, W. T. (1984): Gregarious nesting of a digger wasp as a “selfish herd” response to a parasitic fly (Hymenoptera: Sphecidae; Diptera: Sarcophagidae). *Behav. Ecol. Sociobiol.* **15**: 157 - 160.

Wcislo, W. T. (1986): Host nest discrimination by a cleptoparasitic fly, *Metopia campestris* (Fallén) (Diptera: Sarcophagidae: Miltogramminae). *J. Kans. Entomol. Soc.* **59**: 82 - 86.

Werner, T. K. and Sherry, T. W. (1987): Behavioral feeding specialization in *Pinaroloxias inornata*, the ‘‘Darwin’s finch’’ of Cocos Island, Costa Rica. *Proc. Natl. Acad. Sci. USA* **84**: 5506 - 5510.

Wilson, E. O. (1971): *The Insect Societies*. Belknap Press, Cambridge, MA.

Yanega, D. (1990): Philopatry and nest founding in a primitively social bee, *Halictus rubicundus. Behav. Ecol. Sociobiol.* **27**: 37 - 42.