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ORIGINAL RESEARCH

Nest Architectural Patterns by Three Wasp Species (*Vespa velutina, Polistes flavus* and *Sceliphron formosum*) with Reference to Their Behavior

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Abstract: In the present study, the nest architectural patterns, elemental analysis and their behavior were carried out in three wasp species: *Vespa velutina* (Lepeletier), *Polistes flavus* (Cresson) and *Sceliphron formosum* (Smith) from the different localities of the Mansehra, Pakistan. The *V. velutina* nest was completely closed except for one opening for entry or exit with 1–10 layers of hexagonal cells inside the nest. The nests of *P. flavus* were found among bunches of leaves of trees with 1–5 layers and hexagonal cells same as in *V. velutina*. Nests of the *S. formosum* were pitcher-shaped, found in muddy places, and consisted of 1–10 cells. Social behavior of wasps showed strong foraging, defensive behaviors, pseudo-attack, subsequent erratic flight, wing buzzing, mandibular pecking, abdominal pumping and abdominal twisting with highly developed parental care. It was concluded that the behaviors of these 3 wasp species was highly developed as compared with other insects.

Keywords: elemental analysis, hornet wasp, Mansehra, mud dauber wasp, paper wasp, social behavior

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Introduction

The name wasp applies for 2 paired winged insects of the order Hymenoptera and suborder Apocrita, and is indicated when the insect is neither a bee nor an ant.[1] Some wasps are solid black or dark blue but the most have red, orange, or yellow wings with many markings and stripes are also common. The majority of 20,000 species are solitary, but one family Vespidae includes both social and solitary forms. Social forms include the hornet wasp, *Vespa velutina* (Lepeletier), paper wasp, *Polistes flavus* (Cresson), aerial yellowjacket, *Dolichovespula arenaria* (Fabricius), and bald-faced hornet, *Dolichovespula maculata* (Linnaeus). Solitary forms include potter wasps, *Eumenes fraternus* Fabricius. However, Sphecidae is a cosmopolitan family of wasps with altruistic or solitary behaviors that includes digger wasps, e.g., mud daubers, *Sceliphron formosum* (Smith) and other familiar types that all fall under the category of thread-waisted wasps. Therefore, *V. velutina*, *P. flavus* and *S. formosum* can be compared with reference to architectural patterns and elemental analysis of their nests as well as parental care and social behavior.[2]

In social wasp colonies, there are usually three castes, the egg-laying queens (one or more per colony), the workers (sexually undeveloped females) and the drones (males). Social wasps build nests of a coarse, papery material or masticating wood fibers. In the temperate regions, a colony lasts a single season, with the drones and workers dying in the fall. The mated queens take shelter during the winter, and then in spring they lay eggs and start new colonies. In the tropics, colonies continue indefinitely, dividing when they grow very large.[3] The *V. velutina* found throughout the Northern hemisphere builds a large, round nest of many combs, covered with a paper sheath. In some species this nest is built underground. Wasps begin construction of a nest that may eventually house more than 500 adults. The queen measures the dimensions of the nest with her antennae throughout the continuing nest expansion. By the end of the summer, a large nest contains drones, workers and a number of new queens, which leave the nest to start their own nests in springtime.[4]

Wasps sting to defend themselves or their colony. Stinging involves the injection of protein venom that causes pain and other reactions. Wasps can sting more than once because they are able to pull out their stinger without injury to themselves. When stinging by a wasp or bumblebee occurs, the stinger is not left in the skin. Most people have only local reactions to wasp stings, although a few may experience more serious allergic reactions. Local, non-allergic reactions range from burning, itching, redness, and tenderness to massive swelling and itching that may last up to a week.[5]

Even in monogynous colonies headed by a singly mated queen, worker reproduction of males is inhibited by worker policing.[6] In Vespinae, no queen replacement occurs, and the colonies are monogynous. However, when new queens appear, they leave their nests during the late summer and mate with males. The queens then seek out sites for winter, such as under loose bark, in rotted logs, under siding or tile, and in other small crevices and spaces, where they become dormant. These queens become active the following spring when temperatures are warm. They search for favorable nesting sites to construct new nests. They do not reuse old nests.[7]

Mansehra is located at the Eastern border of the Khyber Pakhtunkhwa (KP), 244 km away from Peshawar and 190 km away from Islamabad. It is bounded in the north by Battagram and Kohistan districts, in the east by Muzaffarabad district of Azad Jamu and Kashmir, in the south by Abbottabad and Haripur districts and in the west by Buner and Shangla districts. This city is a major stop for tourists on the Karakoram highway that leads to China. Diverse fauna of wasps have been found in Mansehra including Braconidae, Chrysididae, Chalcididae, Eurytomidae, Pompilidae, Pteromalidae, Scoliidae, Sphecidae, Sphingidae, and Vespidae.[8] The objectives of the present study were to study differences in the architectural patterns of the nests of three wasp species, their social behaviors, and parental care of their offspring.

Materials and Methods

Observation for architectural patterns of wasp nests

Three species of the wasps and their nests were collected from the different sites: Hazara University (n = 7), Baffa Mera Chotal (n = 5) and Hassa Balakot (n = 6) located in Mansehra, Pakistan. The visits (1 visit at 1 place per day for 3 months) were made in order to observe construction of architectural patterns, and to collect materials.

Elemental analysis

Different nests building material samples (n = 6) were ground into fine powder, kept in sterilized vials

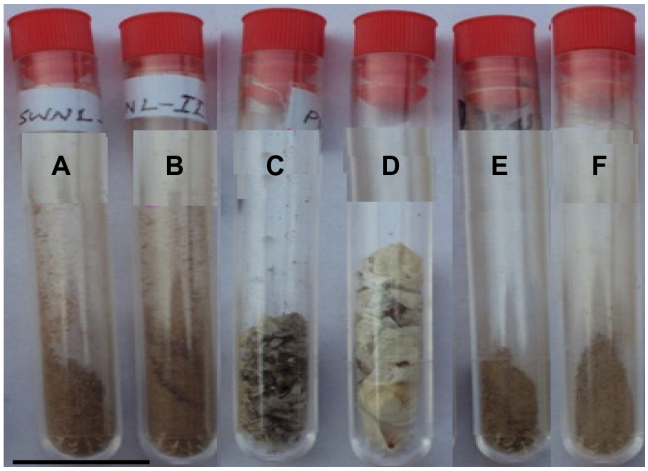

Figure 1. Powder prepared for the XRD analysis of outer and inner part of the nests of the hornet wasp, *Vespa velutina* (Lepeletier): (**A** and **B**); paper wasp, *Polistes flavus* (Cresson): (**C** and **D**); mud dauber wasp, *Sceliphron formosum* (Smith): (**E** and **F**); bars in photographs indicate 30 mm.

and labeled accordingly: outer and inner part of the nest of *V. velutina* (A and B), *P. flavus* (C and D) and *S. formosum* (E and F), respectively (Fig. 1). Samples were analyzed by a gas reaction, which is a chemical transport reaction. These were often carried out in a sealed ampoule to which small amount of transport agent and iodine were added. The ampoule was then placed in a zone oven. This was essentially made up of two tube ovens attached to each other, allowing a temperature gradient to be imposed. Such a method can be used to obtain the product in the form of single crystals suitable for structure determination by X-ray diffraction (XRD).[9]

Observation of social behavior and parental care

The visits (1 visit/1 place/day for 3 months) were made to collect nests of the wasps where an active nest was found. Attempts were made to provoke adults to defend by prodding them with the tip of a stick or a comparable instrument. These visits were carried throughout the colonial cycle. Defensive behavior was videotaped including departures, arrivals, collecting of food, and collecting of nest building materials.[10]

Results

Three wasp species, *V. velutina* (n = 33 workers), *P. flavus* (n = 30 workers) and *S. formosum* (n = 28 workers) were identified from three types of nests collected from Hazara University, Baffa Mera Chotal and Hassa Balakot located in Mansehra, Pakistan.

Comparison of architectural patterns of three wasp nests

We observed wasps to build their nests in a systematic and architectural pattern. It was found stelocyttarous (insect's nest supported by one or more pillars: petiolated) calyptodomus (concealed nest) in *V. velutina* and stelocyttarus gymnodomous (single-combed, unprotected, and open) in *P. flavus*, as previously reported by to Richards and Richards.[11]

Hornet wasp, *Vespa velutina* (Lepeletier)

The spherical nest of *V. velutina* was mostly found attached downward towards the roof of the veranda. The attached portion of nest was flat and continuously increased from only one side. The nest was bounded by a protective layer. Inside, 1–10 layers were found, depending on increasing numbers of wasps. The first layer starts from the upper side by a strong attachment, which led to a layer of hexagonal cells. One main opening was found for entry or exit. Many small tubes like routs were also found in each layer, which led to the main opening. Nests were used for many years until they were destroyed.

Paper wasp, *Polistes flavus* (Cresson)

The nest of *P. flavus* was found among bunches of leaves in the tree branches. Structurally, it was oval-shaped and attached with a minute but strong stalk. It was formed with a single layer of hexagonal cells, which opened directly to the outside. With increasing numbers of wasps, the workers started to build new layers beneath the old ones. They reached a maximum of 5 layers. The oldest layer was the largest, and others gradually decreased in size.

Mud dauber wasp, *Sceliphron formosum* (Smith)

The small pitcher nest of *S. formosum* was made up of mud. It was found in muddy places where building materials were easily available. It has a smooth outer surface attached from the lower side. It contains up to10 cells for larvae. It has one opening on upper side for entry or exit. They close their nest opening up to the maturation of their larvae.

Comparison of elemental analysis of three wasps nests samples

The analysis by XRD shows the amount of elements with descending order in outer and inner parts of nest of *V. velutina*: Ca > K > Al > Mg > Si and Ca > K > Al > Mg, respectively (Fig. 2A and B), as well as for *P. flavus*: Ca > Si > Al and Ca > Si > Mg, respectively (Fig. 2C and D), and *S. formosum*: Ca > Si > K and K > Si > Mg, respectively (Fig. 2E and F). The amount of elements in outer and inner parts of nests was different because three species are living and collected materials from different localities and environments.

Comparison of social behavior of three wasp species

During the present study, there were three social casts found in each wasp species. Social behavior of wasps showed strong foraging, defensive behaviors, pseudo-attack, subsequent erratic flight, wing buzzing, mandibular pecking, abdominal pumping and abdominal twisting. Many workers were seen busy with their duties, ie, collecting building materials, foraging, constructing the nest and caring for offspring. A single queen was present for laying eggs. Many drones were permanently present on duty around the nest. Since their main job is to mate with the queen, males typically die soon after mating, but they showed strong defensive behavior when anything disturbed the nest or the area around the nest.

Hornet wasp, *Vespa velutina* (Lepeletier)

In the opening of the *V. velutina* nest, a number of strong and very active drone wasps were present to prohibit entry of intruder wasps. If they found another species, they attacked and killed them through fighting and eating (Table 1). This behavior was observed repeatedly. The workers have no interest in the drones' activities.

The queen lays five eggs in a small comb protected by several layers of papery material. She forages for wood fibers that are chewed and mixed with saliva to form new layers for her nest.

Paper wasp, *Polistes flavus* (Cresson)

Polistes flavus build their nests among bunches of leaves of trees. Most of the nests are single layered but when the number of wasps increases, they start to build the next layer over the old one and continue to reach 4–5 layers.

Mud dauber wasp, *Sceliphron formosum* (Smith)

Sceliphron formosum build their nests in the mud. Only one couple lived in their nest when it was completed. The female laid the eggs in prepared chambers. Subsequently, they visited the nest from time to time to care their eggs.

Comparison of parental care of three wasp species

During the present study, the parental care of three wasp species was recorded. When the queen found a suitable location to start a new nest with 20–30 cells, wood fibers were chewed and mixed with saliva before the laying of eggs began. The queen created a single cell at the end of a petiole; six more cells were then added around this to create the hexagonal shape of the nest cells. Once the eggs were hatched, wasp larvae were molted within the brood cells. When ready they would spin a silk cap over the top of the cells and then pupate into adult worker wasps. All workers were busy with their duties, collecting building materials, foraging, constructing the nest and caring for offspring. Parental care by workers was observed by introducing a pen camera during video making, and afterward by breaking the nests. Each nest consisted of one queen, who was differentiated via her large size. It was observed that she was paired with one drone, and the rest may have been sterile worker wasps as they were small in size compared with queen, and were never observed to mate with drones. Throughout the summer, new queens and fertile male drones were produced. It was not observed whether each female mated with one or more male. After mating, these new queens hibernated through the winter, and were ready to start a new nest in the spring. Meanwhile, *S. formosum* had only one couple in the nest. When the female lays the eggs, she is responsible to care for the offspring and nest in order to protect them from predators for her whole life span.

Discussion

In the present study, nest building activities, social behavior and parental care were examined in three wasp species, *V. velutina*, *P. flavus* and *S. formosum*.

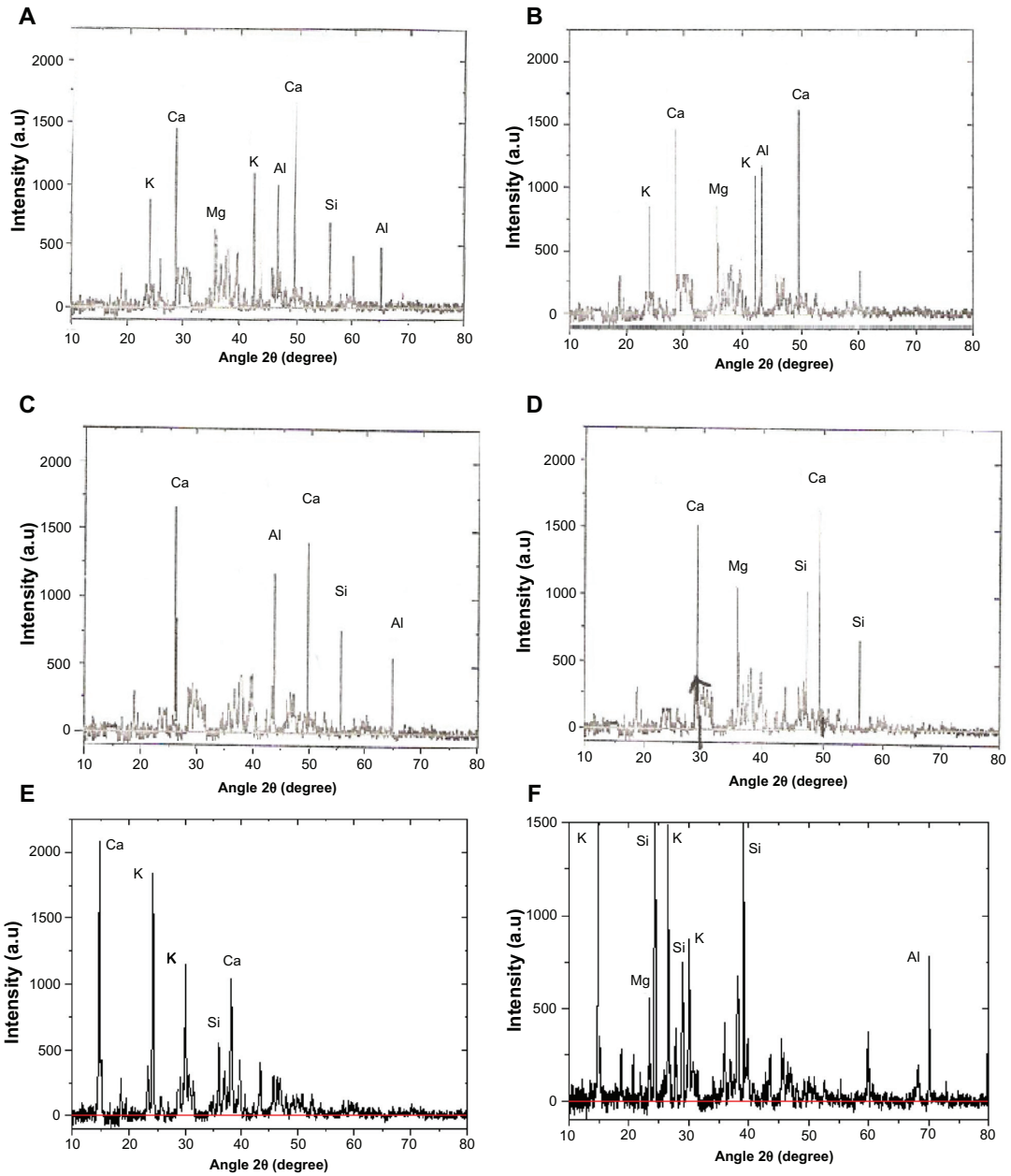

Figure 2. Comparison of elemental analysis by XRD of the outer and inner parts of the nest of the hornet wasp, *Vespa velutina* (Lepeletier) collected from Hazara University: (**A** and **B**); the paper wasp, *Polistes flavus* (Cresson) from Baffa Mera Chotal: (**C** and **D**); the mud dauber wasp, *Sceliphron formosum* (Smith) from Hassa Balakot: (**E** and **F**), located in Mansehra, Pakistan, respectively.

Karsai and Wenzel[12] reported that *V. velutina* showed differences in nest structure and architectural patterns from other wasp species, with nest construction methods as described in Results section. In the present study, each worker wasps constructed nests in different shape, size, architectural patterns and their nest building activities were found to be quite different. The nest building behavior in wasps is very advanced as compared to the other arthropods. The worker wasps collected building materials from a far distance, due to perception of availability of construction materials.

Wilson[9] reported that elemental analysis by XRD was used to determine chemical composition of wasp nests constituents. In the present study, the same technique was used. The major constituents in the nest were calcium, silicon, magnesium, aluminum and potassium. Peaks in the diffractive grams showed different levels of these constituents (Fig. 2). In both studies, calcium was present in the highest

Table 1. In the hornet wasp, *Vespa velutina* (Lepeletier) nest, the drone wasps were present for prohibiting entry of intruder wasps.

S. no.	Number of observations*	Number of males involved	Manner of prohibiting entry of intruder wasps**
1	5	5	Attacking (3)***/killing (2)
2	10	7	Attacking (4)/killing (3)
3	11	11	Attacking (6)/killing (5)

Notes: *Number of observations: were made during different days (8:00 am–5:00 pm) of study periods; **attacking: prohibiting the entry of intruder wasps in the nest through repelling by fighting and killing: intruder wasps died during fighting; ***number in parentheses represents cases of the manner attacking/ killing.

frequency in the constructed materials. Its presence depends upon materials collected from surrounding areas. However, it is responsible for water-resistance and strengthening of the nests, as well as repelling of predators/parasites from the nests.

Foster and Ratnieks[13] observed the colonies of social hymenoptera and reported that male production was undertaken by queens or unmated workers. Karsai et al[12] examined complexity and specialization in task partitioning at both individual and colony levels. Consistent with models of Oster and Wilson,[14] it was predicted that in small wasp colonies, risk tolerance and behavioral flexibility of individual workers would be preferred, whereas wasp species characterized by large colonies should rely upon a high rate of exploration and exploitation of the environment by numerous small specialized worker wasps. In the present study, each nest was found in a different location, demonstrating that each species builds their nest in a unique environment. Some of the nests had small colonies and others had large colonies, reflecting findings reported by Foster and Ratnieks[13], Karsai et al[12] and Oster and Wilson[14].

Matsuura et al[2] reported that the *V. velutina* has the largest colony size (1,500–4,500 cells) among the six subspecies. In each cell, 1–4 eggs are laid by the wasp. In the present study, it was found that nest of *V. velutina* has a number of hexagonal cells, and a step-wise portion are prepared for thousands of eggs to be laid by the queen and number of larvae hatched and came out from the cells with the same manner. One can argue that it is inherited characters that were developed different from race to race.

Hermann and Dirks[15] showed that in *P. flavus*, the heavily sclerotized sting shaft ensured a mechanical penetration into the victim, while the highly specialized venom gland delivers the powerful venomous secretion. For this purpose, an impressive muscular supply surrounded the glands reservoir. Billen and Ito[16] showed that the secretion was forced into the venom gland duct by muscle contraction; it was carried straight through the sting and injected it into the victim. In the southeast Asian *Stenogastrinae*, the secretion of Dufour's gland was used in larval nutrition and nest defense. In the present study, this aggressive behavior of the wasp was clearly found, and authors were stung by the wasp with a similar mechanism as is mentioned above. The reason for such behavior is to protect themselves from external environmental factors, such as the interference of humans, removal of their nests from the houses, and cutting the tree branches where they built their nests.

Rossi and Hunt[17] reported that the wasps collected their food from fields of different crops like rice, cotton, and wheat. Raw materials from the cotton field were used to construct their nests. In the present study, it was observed that the wasps collected their food from different fruit markets. Such materials and foods were easily available in the areas surrounding the wasp nests.

Defensive behavior studied by Eberhard[18] in *P. flavus* appeared to be non-existent during early stages of colony establishment. However, a pseudo attack was elicited from drone wasps upon strong provocation. However, many worker wasps were found to be performing security duty around the nests for protection. Therefore, wasps have a well-developed management system for protection of their nests. One can argue that such behavior indicates that wasps were planning to use their old nests for the next season. During the present study, the aggressive behavior of the wasp was clearly found. The reason for such behavior was to protect themselves among tree branches where they built their nests.

Owen[1] observed a pseudo attack in *P. fuscatus*. The work of Brennan[19] on abdominal wagging in *P. dominulus* has been used to describe the behavior and substrate vibrations of the wasps. In the present study, it was observed that the drone wasps flew nearby around their nests, but not toward the object where nest was found. Thus, being synanthropic wasps, these wasps built their nests in the homes and trees.[20] Litte[21]studied the polistine wasp, *Mischocyttarus mexicanus* and discussed the defensive behavior of the *V. velutina*. In both studies, fast movements of wasps around their nest were found, which showed strong defensive behavior against their enemies. The reason for such behavior was to protect their nest and larvae from foreign disturbance. Eisner[22] studied wasp hunting behavior and Hunt[23] investigated the wasps' foraging behavior. In both studies, wasps hunted their prey in two steps: in the first step, wasps explored the environment until they located and visited potential prey, and in the second step, they attacked and tried to capture the prey. One can argue that wasps hunt prey according to their food requirements. Hunt[23] investigated the wasps' foraging behavior. In the present study, it was observed that hymenoptera modified their flight when they perceived a black spot on a light object. They are attracted to small elements that have a high contrast with their surroundings. Since drones are not generally very active in the colony, their main job being to mate with the queen, they die soon after mating. However, they still played a strong defensive role when something disturbed their nest or was near the nest. In the *V. velutina* nest opening, many strong and very active drone wasps were found to be stopping the entry of other types of wasps. Further studies should be conducted to further examine the role of the male drone wasp.

Conclusion

It was concluded that the social behavior of *V. velutina*, *P. flavus* and *S. formosum* were highly developed as compare to other insects. A high frequency of protection was clearly observed in *V. velutina,* while *S. formosum* showed a low frequency of protection. Reproduction and parental care was unique and isolated from wasp nests. Nests of wasps were different in size, shape, structure and building patterns. The XRD showed that the amount of calcium was highest while potassium was lowest in three wasps nests' constituents.

Author Contributions

Conceived and designed the experiments: FP. Analyzed the data: FP, MS. Wrote the first draft of the manuscript: FP, MS. Contributed to the writing of the manuscript: FP. Agree with manuscript results and conclusions: FP, MS. Jointly developed the structure and arguments for the paper: FP, MS. Made critical revisions and approved final version: FP. All authors reviewed and approved of the final manuscript: FP, MS.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

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