Defensive warning behavior expressed by three species of polistine wasps

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Abstract

Although expressions of warning intruders in colonies of polistine wasps appear to vary somewhat with species-specific defensiveness, the type of intrusion, age of the colony, age and type of the nest occupants, and degree of colony homeostasis, they often share commonly expressed forms of behavior when warning both invertebrate and vertebrate intruders in the vicinity of the nest site. Warning behavior is not associated with most of the home range but with a nest yard, an area within visual range of the nest. The distance from the nest at which stinging is demonstrated is defined as a sting threshold point, which varies among different species and at different periods in the annual cycle. Aposematic coloration and pattern, expressed to varying degrees in different species and sexes, are demonstrated passively but play an active role in defense when combined with warning displays. While males do not engage in active defense, they play a more passive defensive role, and sometimes possess stronger aposematic patterns than those of females. They also engage in a low degree of overt warning expressions.

Key Words: warning behavior; social wasp defensive behavior; aposematic coloration; nest yard; sting threshold

Resumen

Mientras que las expresiones de advertencia en las colonias de avispas polistinas parecen variar algo con actitudes defensiva propias de cada especie, el tipo de intrusión, edad de la Colonia, edad y tipo de ocupantes del nido y el grado de homeostasis de la colonia, a menudo comparten formas comunes de comportamiento de advertencia ante intrusos que se acerquen al nido ya sean invertebrados o vertebrados. El comportamiento de advertencia no está asociado con la mayoría del área de vida (home range) sino con el "patio del nido," la adyacencia inmediata visual al nido. La distancia del nido a la cual puede suceder la picadura se define como el umbral de la picaura, la cual varía entre diferentes especies y en diferentes períodos del ciclo anual. Coloración aposemática y patrón, expresado en diversos grados en diferentes especies y sexos, se manifiestan pasivamente, pero juegan un papel activo en la defensa cuando se combina con el comportamiento agonístico. Aunque los machos no participan en la defensa activa, ellos juegan un papel defensivo pasivo y a menudo poseen patrones aposemáticos más fuertes que los de las hembras. También participan en bajo grado en manifiestas expresiones de advertencia.

Palabras Clave: comportamiento de advertencia; comportamiento defensivo de avispa social; coloración aposemática; patio de nido; umbral de picadura

As stated by Anderson (1984) and Judd (1998), nests and the location in which they are established play a crucial role in the life histories of many animals. A nest, which is a characteristic feature in colonies of social insects, centralizes the location of a colony's brood, providing an efficient way to care for an ever-increasing number of offspring during the nesting season. However, a major cost in possessing a centralized nest includes the potential of losing part of or an entire colony to predation or parasitoidism (Jeanne 1975; Anderson 1984; Starr 1985, 1990; Kukuk 1989; Macom & Landolt 1995; Hansell 1996).

Vespine wasps (Vespidae: Vespinae) typically construct multi-tiered, phragmocyttarous nests (layers of brood comb are attached at their periphery to an envelope) in which several outer paper layers conceal the inner cells and offer protection for the brood against direct intruders. The 2 Florida *Vespula* species even use subterranean chambers to further protect their colonies from intruders. Social wasps that construct stellocyttarous (nests attached by 1 or more pedicels to a substrate), single-tiered, gymnodomous nests (lacking an envelope) in which the cells are clearly exposed (Vespidae: Polistinae) are vulnerable especially to intrusion by potential predators and parasitoids (Hermann 1984).

There are 10 species of social wasps in the genus *Polistes* (subgenus *Aphanilopterus*) and 1 species of *Mischocyttarus* (subgenus *Monocyttarus*) in Florida (Carpenter 1996): *Polistes annularis* (Linnaeus), *Polistes apachus* Saussure, *Polistes bahamensis* Bequaert and Salt, *Polistes bellicosus* Cresson, *Polistes carolina* (Linnaeus), *Polistes dorsalis dorsalis* (Fabricius), *Polistes exclamans* Viereck, *Polistes fuscatus* (Fabricius), *Polistes major major* Palisot de Beauvois, *Polistes metricus* Say, and *Mischocyttarus mexicanus cubicola* (de Saussure) (all Hymenoptera: Vespidae). All construct stellocyttarous, single-tiered, gymnodomous nests in which the cells are clearly visible.

Both predators and parasitoids attack the nests of these types of wasps. Jeanne (1972) initially reported on a number of bird species that attack polistine wasp nests, and other reports have mentioned assorted birds and rodents, as well as a number of types of predatory ants (Gibo 1978; Turillazzi 1984). While the act of predation by vertebrates is rarely observed, Henriques and Palma (1998) witnessed predation by curl-crested jays (*Cyanocofrax cristatellus* F. Boie; Passeriformes: Corvidae) on a nest of *Apoica pallens* (Fabricius) (Hymenoptera: Vespidae) in the Brazilian savannah. Most reports on nest predation are based on

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finding damaged nests. Since this report does not address parasitoid attacks on nests, it will not be discussed here.

Because vertebrate predation represents a serious threat to the homeostasis of polistine colonies (Jeanne 1975; Gibo 1978; Hermann 1984; Turillazzi 1984; Starr 1985, 1990; Bruschini et al. 2005), all species have developed behavioral expressions that warn intruders to stay away from the nest or suffer the consequences of attack. Expressions of warning behavior have been recorded for a number of polistine species (Hermann 1984), including *P. annularis* (West Eberhard 1969; Hermann & Dirks 1975), *P. fuscatus* (Jeanne 1975; Judd 1998), *M. mexicanus cubicola* (Hermann & Chao 1984), and *Mischocyttarus cerberus* Ducke (Hymenoptera: Vespidae) (Togni & Giannotti 2010).

Visibility of the open nest provides ample opportunity to record and describe forms of defensive behavior when the nest is approached by an intruder. Continued research on defensive behavior may enable the scientific community to accumulate enough information on warning behavior as expressed by polistine wasps to have a library of common and species-specific expressions of defensive behavior at its disposal. Such studies will add to an understanding of wasp defensiveness, the totality of features used by a species to defend its nest and inhabitants. It is to this end that we present this investigation of warning expressions demonstrated toward vertebrate intrusion in 3 polistine wasp species in South Florida, and comparatively discuss their importance, commonality, and differences.

Materials and Methods

Observations on defending nests of *P. dorsalis*, *P. major*, and *P. metricus* (the 3 most common species of *Polistes* in our area) were carried out during the spring and summer mo of 2014, 2015, and 2016 in Lee (Ft. Myers) and Hendry (Clewiston & LaBelle) counties, Florida, USA.

A Sony Handy Cam (model DCR-SX45/SX65/SX85; Sony Group Corp., Konan, Monato, Tokyo, Japan) digital video camera with extended (70×) zoom and built-in USB was focused initially on each nest face prior to nest disturbance to record what might be construed as normal colony behavior.

Using a camera with a powerful zoom lens allowed us to place the camera outside the nest yard (defined below). Following a short sequence of undefended (routine colony) activity, we approached each nest head on to determine the size of the nest yard. Video sequences were subsequently watched repeatedly to observe all forms of warning behavior.

Wasps on each nest were subsequently stimulated to defend by using a 3.5 m metal pole fitted with foam plastic on 1 end which was covered with a black cloth. Depending on the number of nests we could find, several trials were run to be sure we captured all expressions characteristically demonstrated during nest defense.

As a preliminary step in viewing defensive behavior, a defensive zone which we describe for the first time as a nest yard was determined by measuring the distance from the nest to the spot at which the defenders first responded to nest intrusion. We refer to the point within the nest yard at which workers begin to sting as the threshold point.

Results

Nests of the 3 species in this investigation were located primarily on wooden buildings and other structures except concrete. High temperatures at that time of yr were from 27 to 32 °C, and night-time temperatures dipped to 16 to 21 °C. Nests varied in size from a few cells during the nest-founding period to relatively large ones (hundreds of cells) during the ergonomic period.

Without adequate cold temperatures to drive reproductive females into hibernation, polistine species in South Florida often were found to remain on the nest throughout winter, and new nests were founded at various times during the warmer mo. Consequently, determination of pre- and post-emergence periods was found to be dependent more upon colony size, the presence of silken caps, and on the types of adults inhabiting the nests.

Nest Yard

The area around a nest which we refer to as the nest yard is defined as the area in which defensive expressions are demonstrated toward intruders (Fig. 1). The size of the nest yard varies between species but appears to be relatively constant within a species, regardless of nest size as long as workers are defending (i.e., it is within the ergonomic part of the nesting cycle). As indicated by data in Table 1, the size range of nest yards (distance from the nests) for the species studied were as follows: *Polistes dorsalis* – stable at 252 cm; *Polistes major* – 122 to 140 (mean of 134 cm); *Polistes metricus* – 5 to 173 cm (mean of 91 cm).

First Sign of Defense

The first sign that wasps are aware of an intruder's presence (the outer edge of the nest yard) was twitching of the prothoracic tarsi (Table 2). After repeated viewings of the recordings, twitching became easy to see, but it was difficult to detect by simply viewing through the camera lens without repeated viewings. There is a range of difficulties in recognizing prothoracic tarsal twitching among different species. It is detected easily, for instance, on nests of *P. metricus*

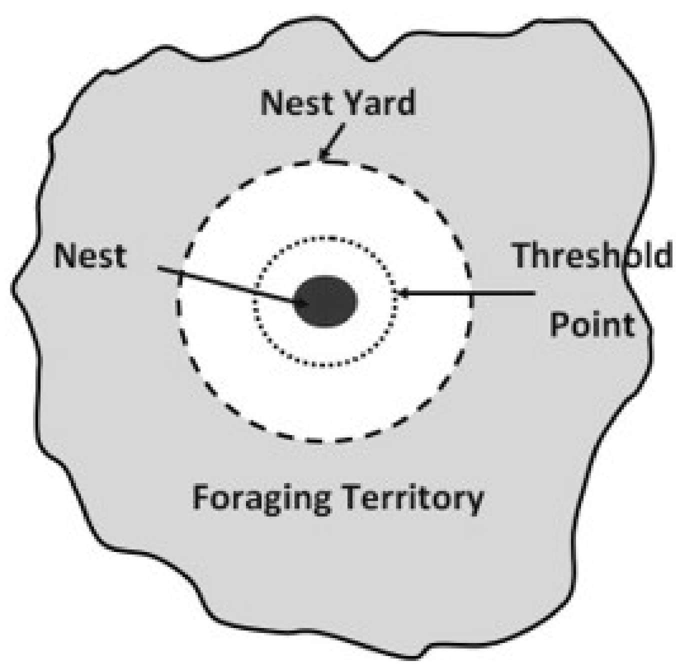

Fig. 1. Diagram of foraging area, nest yard, and sting threshold point. Foraging territory is the area surrounding the nest in which colony workers scout for and obtain their prey, paper, and other resources. Outside the nest yard, they remain non-defensive unless they are subjected to personal threat. The nest yard includes the area around the nest but outside of the sting threshold point in which warning behaviors are expressed. An intruder who approaches the nest in the area within the sting threshold point is likely to experience stinging behavior.

Table 1. Summary of nest yard, point of threshold for stinging*.

Nest number	Nest condition
Polistes major	nest yard size 122 to 140 cm (mean 134 cm); sting threshold distance 0 to 30 cm (mean 10 cm)
Pmaj1	10 cm nest; healthy, second round of caps, near center of eave, about 30 females on nest
Pmaj2	5 cm nest; healthy, small, with few caps, south side of barn; nest lost during investigation
Pmaj3	5 cm nest; nest lost during investigation
Pmaj4	Lone female; nest lost during investigation
Pmaj5	29 cells with larvae and eggs; 4 caps; 3 other cells with larvae; no apparent parasitism; 3 females
Pmaj4	Lone female; 11 cells had silk rims; 1 cap; queen on nest face; on wafer board; turning abdomen, pumping abdomen, frantic leg movement; checking cells; cleans antennae and face
Pmaj7	Lone female (queen); sawhorse. This nest was lost by 12 Jun
Pmaj8	9 cm long × 6.5 cm wide (oval shape); 152 cells; 10 closed caps; at least 32 cells with a fringe of silk, left from emerges; single robust petiole; no sign of parasitism except small holes on top of nest. Nest lost after videotaping
Pmaj9	6.3 cm nest; beneath air conditioner on vacated mobile home; 141 cells; 4 caps; nest was defunct upon collection on 4 Jul
Polistes dorsalis	nest yard size 252 cm (mean 252 cm); sting threshold undetermined
Pdor1	10 cm nest; 21 cells; 4 caps; 3 cells with large larvae; 3 adult females; no males
Pdor2	4 cm nest; 54 cells; 7 caps; some moth parasitism; 2 females, 1 of which did not have an aposematic face
Pdor3	3.2 cm nest; 51 cells; no adults; no caps; nest was defunct at collection
Pdor4	8.4 × 6.6 cm nest; 197 cells; 4 caps; parasitized by moths; 1 female and 1 male
Polistes metricus	nest yard size 5 to 173 cm (mean 91 cm); threshold distance
Pmet1	2.5 cm nest; 2 females on back porch; 13 cells; 4 caps; parasitized by moths; nest was defunct when taken on 4 Jul
Pmet2	8 × 5 cm nest inside van on rubber tube; 81 cells; 10 caps; no apparent parasitism; 9 adult females; no males
Pmet3	5.8 cm nest on vertical wall panels in van; 63 cells; 12 caps; no apparent parasitism; four adult females; no males

*Some nests were lost during investigation due to homeowner control, resulting in some data loss of cell number, number of caps, and number of adults on nest.

because of their relative non-aggressiveness, but it was not as obvious in other species because of the predominance of other warning behaviors that were occurring at the commencement of and during nest defense.

The first obvious signs of defensive expressions in all species were wing and antennal raising and spreading which were visible plainly even without the use of a camera. Since these 2 movements are simultaneously expressed, they are considered as a single composite expression. Although antennal raising and spreading also are expressed as a composite investigative behavior, wing raising and spreading upon provocation are designated as the first true signs of warning. With increasing provocation, wing raising and spreading led to wing flipping, fluttering, buzzing, and flight (Table 2). During wing spreading, flipping, and fluttering, the longitudinal fold in the wings which characterizes vespids was maintained. During the latter 2 expressions (buzzing and flight), the wings are unfolded.

Table 2. Warning expressions for 3 species of polistine wasps*.

Behavior	*P. dorsalis*	*P. major*	*P. metricus*
Cell checking	X	X	X
Cleaning body	X	X	X
Facing intruder	X	X	X
Non-stinging flight	X	X	X
Hiding behind nest	X	X	
Investigative behavior	X		
Jerking*	X	X	
Pumping abdomen*	X	X	X
Sting pointed toward intruder*	X	X	X
Raising forelegs*	X	X	X
Spinning*	X		
Tapping gaster on nest*			
Tarsal twitching*		X	X
Walking around nest*	X	X	
Wing buzzing	X	X	
Wing flipping*	X		
Wing fluttering*	X	X	
Waving foreleg*	X	X	X
Wing raising*	X	X	X

*Those expressions accompanied by an asterisk are considered warnings. Those without an asterisk are considered ancillary expressions of warning behavior.

At times, wing raising and spreading was preceded by or simultaneously expressed with turning the body toward and facing the intruder, another investigative maneuver but one that flaunts the aposematic facial pattern. Since wing raising and spreading was a consistent marker of intruder detection and is defined easily, it was what we used to determine the outer border of the nest yard (Fig. 1). Other warning expressions ensued as the colony was further provoked.

Some degree of partial wing raising and spreading was detected on nests that were not provoked. Often they became momentarily expressed to a higher degree when workers returned from foraging, and workers often rushed to that individual, slowing aggressive expressions upon recognizing the incomer. However, under provocation, wing raising and spreading was the most persistently evident expression of intrusion.

As examples of the degree of raising and spreading, determined by measuring wing angles at various times during provocation, *P. major* queens showed a range of 58 to 83° between wings, with a mean value of 72°, and workers showed a range of 81 to 163°, with a mean of 104°. Thus spreading was greater considerably in workers than in queens, indicating a greater role in defensiveness.

In *P. metricus*, wing spreading in workers had a range of 32 to 170°, with a mean of 82°. Queens' range of spreading was 42 to 47°, with a 44.5° mean. Again, spreading by workers was greater.

In *P. dorsalis*, the range for wing spreading in workers was 41 to 85°, with a mean of 63.4°. Wing spreading in queens was of short duration and difficult to measure. Even males of this species spread their wings to a 60° angle. It appeared that 1 of the reasons workers didn't spread the wings more in this species is that as soon as the maximum spreading was reached, they often began to flip, flutter, and buzz them, pointing out that they were more aggressive defenders.

RAISING FORELEGS

Raising the forelegs (often along with raising the body) typically was expressed immediately following wing raising. As soon as the legs are raised, 1 or both forelegs most often demonstrate a waving motion. Under increased provocation, both forelegs were waved sporadically and rapidly. Under even stronger provocation, we have noticed occasional waving of the posterior legs. Some polistines have various degrees of aposematism associated with their legs, and being flaunted, they may enhance warning expressions (e.g., leg waving) against intruders.

WING FLIPPING

We have recognized 3 different forms of defensive wing movement beyond wing raising: wing flipping, wing fluttering, and wing buzzing, which are expressed in this order with increasing provocation (see flow chart, Fig. 2). Wing flipping, like wing fluttering, is carried out with the wings folded longitudinally, but it is expressed as a single or couple of quick flips, whereas fluttering is more prolonged. Wing flipping follows wing raising when some degree of provocation is continuously used to threaten social wasps. Wing fluttering and wing buzzing follow wing flipping as provocation increases.

WING FLUTTERING

Wing fluttering is expressed as a series of wing flips while the wings remain longitudinally folded. They were demonstrated consistently on nests of *P. dorsalis* that had been provoked beyond simply approaching the nest.

WING BUZZING

It is important to distinguish between wing fluttering and wing buzzing because they are distinctly different warning expressions, and they (like flipping and fluttering) are demonstrated under different degrees of provocation. Wing buzzing is a rapid movement of the wings that have been unfolded, as if using them in flight. Buzzing requires strong provocation, such as touching the nest or repeated pounding on the structure which supports the nest. Under strong provocation, wing buzzing in *P. dorsalis* often was accompanied by spinning and jerking movements of the body.

Wing buzzing for the purpose of expressing warning is similar to fanning, which typically is carried out on a nest during the summer mo to cool the nest area. Depending on the location of the nest, fanning may be expressed many times during a recording session, and thus an observer must be careful to distinguish between the 2 types of behavior. Buzzing is a defensive expression.

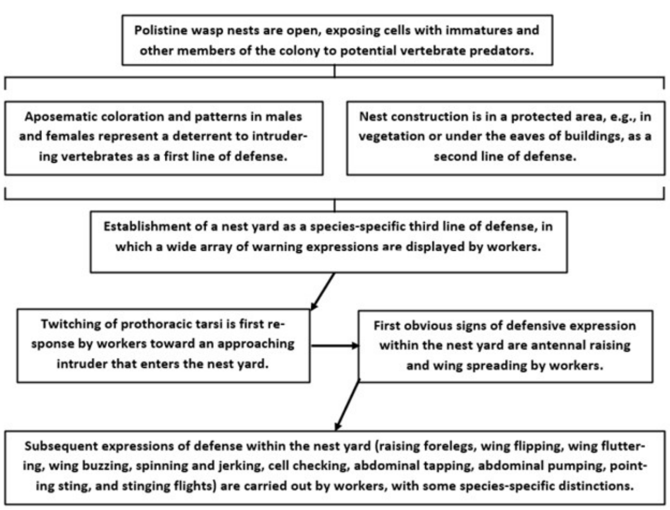

Fig. 2. Flow chart of defensive behavior expressed by polistine wasp workers toward vertebrate intrusion, as determined in this research and other investigations (Hermann 2017; Hermann & Dirks 1974). As initial lines of defense, aposematic coloration and pattern, along with nest location, offer some degree of colony protection. Once an intruder enters the nest yard, warning expressions are displayed by workers. Size of the nest yard and the stinging threshold point generally are species specific. Following the initial pretarsal twitching and the more obvious antennal raising and wing spreading, a wide array of defensive expressions ensue, terminating in stinging flights. There also are some interspecific differences in the display of certain warning expressions, as discussed in the text.

SPINNING AND JERKING

A certain amount of jerky behavior is expressed by many polistine species under strong provocation. As an intruder approaches the nest, wasps generally move forward toward provocation, but when the provocation device is pushed closer to the nest, defending wasps generally jerk the body backward, and subsequently go through the act of moving forward and backward repeatedly. The spinning and jerking behavior described here are new behaviors that we have not seen prior to this study. They were expressed together and under strong provocation, and they were noticed only in *P. dorsalis*. This is not to say they are not expressed in other species, and thus they should be looked for in future studies.

CELL CHECKING

Cell checking was a common behavior expressed by many adult wasps after wing raising on a relatively non-defensive, young nest. Under provocation, it was carried out in a more intense fashion and mostly by the queen and mature workers on larger nests during the ergonomic nesting period. Intensive cell checking often accompanied what Togni and Gionotti (2010) referred to as "restless walking" in colonies of *M. cerberus* and possibly what West Eberhard (1969) referred to as "aggressively darting" in colonies of *P. fuscatus*.

Although queens are non-defensive in terms of stinging, they do demonstrate some of the other expressions, e.g., abdominal pumping and pointing the sting toward the intruder. With continued intrusion, queens generally spent some of their time behind the nest (Table 2), away from intrusion. In colonies with numerous defenders, cell checking increased in intensity most often following the cessation of a period of provocation.

ABDOMINAL TAPPING

Abdominal tapping reported by recent researchers (Jeanne 1975; Togni & Gionotti 2010) appears to be associated with defensive marking chemicals released from abdominal sternal glands (Hermann & Dirks 1974). This behavior is expressed at an accelerated rate when provocation increases. Another behavior that we have referred to as tail-wagging, as done mostly by queens and cofoundresses in pleometrotic species (having a queen and 1 or more cofoundresses) and the queen in haplometrotic species (having a queen and no cofoundresses), also appears as though the wasps are touching their abdomen to the nest and may be construed as similar to abdominal tapping.

ABDOMINAL PUMPING

Slight abdominal pumping often is seen on a non-defensive nest. More vigorous abdominal pumping, however, was expressed upon strong provocation. While other authors have suggested that such pumping enhances the release of defensive pheromones and allomones (Jeanne 1975; Togni & Gionotti 2010), there is evidence that it also increases the oxygen supply to the body, especially the wing muscles (see discussion).

POINTING STING

Pointing the sting toward the intruder is seen readily in all polistine colonies when they are provoked. While it is part of their defensive display, it may be involved in the release of venom or defensive pheromones, although we did not witness droplets of any liquid exuding from the sting tip during either sting pointing or abdominal pumping.

NON-STINGING FLIGHTS

Non-stinging flights from the nest (flying without stinging) often were an escape tactic which was expressed commonly when intrusion was persistent. Nevertheless, it does function as a strong warning. Since dark-eyed workers were reluctant to indulge in stinging, they expressed some on-nest displays but spent much of their time hiding or flying from the nest. Non-stinging flights have been reported for other polistine wasps, especially 4-d-old workers (e.g., *M. cerberus* by Togni & Gionnotti [2010], and other species by Post et al. [1988]).

STINGING FLIGHTS

Most nest occupants that left the nest during provocation did not sting. They either flew non-stinging flights and immediately returned to the nest, or flew off to return later. This feature of defending varies with each species. *Polistes dorsalis* and *P. fuscatus* were relatively quick to leave the nest to sting, and they further engaged in searching or investigative behavior, as if looking for the nest intruder, the latter species expressing more searching than the former; *P. metricus* and *P. major* demonstrated less searching behavior.

Discussion

DEFINING NEW TERMS

We define defense as any behavior employed to warn or deter an intruder from entering the vicinity of the nest. All forms of defensive behavior listed in this paper are expressed within an area that we define as a nest yard (Fig. 1), an area which is within the visual range of nest defenders, in which defensive behaviors are expressed toward an intruder. Nest yard is comparable to territory when the latter is strictly defined as an area in which a type of intraspecific or interspecific competition is expressed in an attempt to keep other organisms from a specific space that is defended as territory (slight modification of Alcock's [2001] original definition). The threshold point for demonstrating the act of stinging is located within the nest yard.

WARNING PARAMETERS

Warning expressions (keep-out signals of Ehrlich et al. [1988]) vary somewhat, depending on whether they result from vertebrate or invertebrate intrusion, and they are performed by different females on the nest, based on what the intrusion may be. Invertebrate intrusion is responded to primarily by queens and cofoundresses (fertile but less dominant females) (Hermann 1984), while vertebrate intrusion is responded to primarily by the worker force, during which the act of stinging generally follows (Jeanne 1975; Hermann 1984, 2017; Turillazzi 1984).

Forms of warning behavior are expressed variously by different species (Table 2). As a measure of colony defense, for instance, some species are more aggressive than others. We perceive species like *P. exclamans*, *P. fuscatus*, and *P. dorsalis* as relatively more aggressive initially than *P. metricus* and *P. major*. In Florida, they would rate higher on a defensive scale (including both warning behavior and readiness to sting). In other categories, they may be placed a little differently in a defensiveness scale, rating at a higher position than *P. metricus* but possibly lower than *P. major*, based on the presence or absence of aposematic coloration.

However, the degree of defensiveness expressed is influenced by other parameters, as well as species-specific differences in aggressiveness and appearance, e.g., investment size, seasonal variation, location of the nest, and health of the colony. A library of defensiveness may even include species-specific differences in sting hemolytic reaction, description of sting reaction and sting pain, as elaborated upon by J. Schmidt and C. Starr (Starr 1985).

Some wing-related expressions, along with abdominal pumping, may not simply represent an increasing indication of irritation to the wasps, requiring stronger forms of warning; they also may represent a more utilitarian service for the wasps by supplying oxygen to the wing-muscle mitochondria, preparing them for their most critical defense, which is flying from the nest to sting.

DEFENSIVE EXPRESSIONS

Seventeen of the expressions demonstrated during intrusion in our study (those marked with an asterisk in Table 2) were considered warnings, including facing the intruder, investigative flight, jerking, pumping of the abdomen, sting pointed toward intruder, raising forelegs, spinning, tapping gaster on nest, tarsal twitching, walking around nest, wing buzzing, wing flipping, wing fluttering, waving forelegs, and wing raising and spreading. A generalized walking around the surface of the nest and cell checking showed concern for brood during intrusion, but they were not part of the warning display. However, an elevated excitedness while walking around the nest, often accompanied by cell checking, raised wings, and investigations of one another, accompanied other warning expressions.

DIFFERENCES IN DEFENSIVE INTENSITY

Nest defense increases in intensity throughout the ergonomic season in response to a growing investment in offspring and an increase in emerging adult defenders (Hermann & Dirks 1974; Hermann 2017). Queens and cofoundresses are non-defensive relatively toward vertebrates (except for expressing certain warning behaviors, e.g., leg waving and wing raising, following periods of provocation). Thus, it was possible to determine the limits of a nest yard. However, a stinging threshold point (the point at which workers commence stinging) for queens virtually is non-existent.

Based on these data, *P. dorsalis* demonstrates the highest degree of defensiveness toward intrusion of the species studied, and *P. metricus* appeared to be the least defensive, the threshold distance for the latter species being in the immediate vicinity of the nest (Table 1). Once the threshold for *P. dorsalis* was breached, stinging ensued, sometimes immediately, although a significant degree of confusion (flying away from the intruder and hiding) were displayed more often. While quite active in displaying warning behaviors during provocation, *P. major* was non-defensive relatively when compared to *P. dorsalis*.

While colony members of *P. metricus* appeared relatively docile during provocation, they appeared to remember intrusion, and 2 stings were received on d and wk following provocation, even though the colonies were not directly provoked on the d of the stings. Perhaps our comment on remembering requires a more substantial support. A person frequenting the vicinity of the nest never had been stung before this investigation, and none of us had been stung during provocation. However, the worker and 1 of us had been stung within 2 wk after threatening them. Memory of social insects pertinent to defense appears to be lacking in the literature.

A significant amount of energy is required to carry out warning behaviors on the nest, especially when they involve rapid movements of the body and wings. Considering abdominal pumping, investigators have found that it facilitates the movement of oxygen through the body. According to Weis-Fogh (1964, 1967), whereas there is no need for increased respiratory movements in small insects because the rate of diffusion of oxygen and carbon dioxide is adequate even during flight, when the thorax is more than a few mm in diam, the primary tracheae or the air sacs which supply the wing muscles must be ventilated. Apart from various auxiliary mechanisms cited by other investigators, respiratory movements include abdominal movements which we refer to as abdominal pumping.

COMPARING DEFENSIVENESS IN NEWLY EMERGED AND OLDER WORKERS

Some reports in the literature point out that dark-eyed wasps are poor defenders. In the publications of Chao (1979) and Chao and Hermann (1983), it was determined that the eyes of newly emerged adults (the dark-eyed adults seen on the nest) are not yet fully developed. Although their compound eyes commence development even prior to spinning their silken caps, they do not complete their development until a number of d after emergence. While they may engage in on-nest expressions of warning, being part of the main defenders and leaving the nest to sting generally is not a viable option as a first line of defense. Thus, their defensive expressions are somewhat limited, and leaving the nest is an option only under strong provocation. Of the other adults on the nest, workers with mature eyes are the most defensive members of the colony toward predator-like intruders, while queens and cofoundresses are relatively non-defensive toward vertebrate provocation. Other researchers have found that queens, cofoundresses, and newly emerged workers are more attentive to their cells and offspring, especially in terms of defense against invertebrate intrusion (e.g., ants and parasitoids) (Burks 1971; Gillaspy 1973; Krombein et al. 1979; Reed & Vinson 1979; Strassmann 1981; Lutz et al. 1984; Macom & Landolt 1995; Giray et al. 2005; Togni & Giannotti 2010).

SEQUENCE OF DEFENSIVE EXPRESSIONS

Polistes dorsalis was the most defensive species in our investigation, and thus it is a useful species for us to describe the sequence of defensive expressions as provocation increases: Wing and Foreleg Raising → Foreleg Waving → Abdominal Pumping, Abdominal Tapping and Pointing of the Sting → Wing Flipping → Wing Fluttering → Wing Buzzing → Spinning and Jerking. This sequence is expressed as a flow chart in Figure 2. Wing and foreleg raising and spreading took place as an intruder entered the nest yard. The other expressions were demonstrated when further provocation was used and increased. Non-stinging flights came about at any time during provocation, depending on the type of nest occupant and nest investment available at the time. Stinging flights occurred either upon rapidly approaching the nest or under continued provocation.

CONSIDERATIONS FOR FURTHER STUDIES

Based on this and earlier studies of defensive behavior, we feel that the following defensive features should be considered standard topics in defining species-specific defensiveness: (1) assortment of warning expressions (as seen in Table 2), including those most often expressed and the sequence of expression, all of which should be clearly defined; (2) measurement of nest yard; (3) measurement of sting threshold point; (4) colony investment, including nest size, number of cells and production of offspring; (5) time of yr; (6) the type of nest occupant or occupants that are defending; (7) the health of the colony (whether it is parasitized or has been preyed upon); (8) readiness to sting and percentage of stinging individuals that leave the nest; (9) searching behavior when leaving the nest; (10) sting reaction (both hemolytic reaction, lesion description, and pain associated with the sting); (11) aposematic coloration in both males and females; (12) location and orientation of the nest; and (13) where in the country or countries the nests are studied. Other researchers may want to add to these features or modify them. Such a comprehensive assessment of defensiveness would be based on defensive features throughout the colony cycle.

IMPORTANCE OF APOSEMATIC COLORATION AND PATTERNS

Aposematic coloration is 1 of the features of defense that often is ignored in studies; yet, we believe this feature is an intricate part of colony defense in many species because it represents a passive cue which allows a predator to identify the wasps as a potential threat. *Polistes metricus* is relatively non-aposematic and relatively non-defensive when compared to *P. dorsalis* and *P. fuscatus*, but it is a large wasp and most likely gains some protection from its size. *Polistes major* is large and brightly aposematic, and it is relatively non-combative when slightly provoked. Some species, like *P. exclamans*, *P. fuscatus*, and *P. dorsalis*, are smaller but aposematically colored and, along with their more intense forms of defense, no doubt are able to keep certain potential intruders at bay, although there is a point beyond threshold at which a persistent predator may cause defenders to leave the nest.

According to Prudica et al. (2007), there is considerable empirical support that conspicuousness promotes the effectiveness of the aposematic signal, and that conspicuous prey are detected sooner and aversion learned faster by a predator as compared with cryptic prey. They also state that predators also retain memory of the aversion longer when prey is conspicuous.

Aposematism is expressed passively by polistine wasps on a non-defensive nest. On a defensive nest, aposematic coloration becomes more of an active form of defense when it is flaunted (flashed and made more evident) during abdominal pumping, raising and spreading the wings, wing flipping, wing fluttering, wing buzzing, waving the forelegs, making erratic jumping and circling movements around the nest, and turning the sting toward the intruder.

In certain species, males are just as aposematically colored as females and sometimes more so, and thus they represent part of the colony's passive defense system. Males of *P. dorsalis* in this area of the US, for instance, often have a brightly colored U-shaped facial marking that females do not possess. Males of other species may not be so brightly colored.

We have seen males of *P. dorsalis* flaunting their aposematic faces, briefly approaching an intruder, and raising and spreading their wings during early provocation, but upon increasing provocation, they move to the back of the nest and point their faces toward the intruder. Togni and Gionnotti (2010) point out the low frequency of alarm behavior expressed by *M. cerberus* males (4.8%). Aposematic coloration in the facial area of some males may add to their efficiency as a defending entity. It is one of the most prominent features an intruder sees when approaching the nest and should be considered as part of the polistine defensive arsenal.

MOST IMPORTANT TOOL FOR OBSERVING DEFENSIVE EXPRESSIONS

A video camera proved to be an invaluable tool to use in recording defensive behavior. By repeatedly watching video recordings made during this investigation, we found that we were able to notice behavioral expressions that were difficult to identify without watching the recordings repeatedly.

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