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SHORT COMMUNICATIONS

JUVENILE *NEPHILA* (ARANEAE, NEPHILIDAE) USE VARIOUS ATTACK STRATEGIES FOR NOVEL PREY

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ABSTRACT. *Nephila clavipes* (Linnaeus 1767) and *N. pilipes* (Fabricius 1793) juveniles exposed to a novel and potentially dangerous prey item frequently attack using thrown silk. To quantify the frequency with which *N. clavipes* opt to use thrown silk, naïve hand-reared small *N. clavipes* juvenile females were observed attacking a new prey type, stingless bees. Repeated exposure to the stingless bees suggests that the spiders incorporate prior experience into prey attack strategies, as experienced spiders attacked using the more usual *Nephila* long-bite.

Keywords: *Nephila clavipes*, *Nephila pilipes*, wrap-attack, prey capture, learning

Orb-web building spiders utilize a range of tactics to capture insects that are restrained in the web, and the choice of tactic varies among species and with prey type (Robinson & Mirik 1971; Robinson & Robinson 1976; Eberhard 1982; Japyassú & Viera 2002). One prey-capture tactic, throwing silk with leg IV, is widely used in phylogenetic analyses of the families of entelegyne spiders (e.g., Sharff & Coddington 1997; Griswold et al. 1999; Agnarsson 2004). In these analyses, the family Nephilidae (previously a tetragnathid subfamily Nephilinae; Kuntner 2006) was assumed to lack this behavior and to attack all prey by biting (Robinson & Robinson 1976; Eberhard 1982; Kuntner 2005a, 2006, 2007) which, since the wrap attack is synapomorphic for the orbicularians (Griswold et al. 1999; Agnarsson 2004), made this a secondary loss in the Nephilidae. This character assignment was based upon observations of adult female *Nephila* species. However, failure to observe attacks using thrown silk in diverse web-building spiders may reflect limited studies, either with only mature animals or with prey that are small relative to the spider's size. Here, I report juvenile females of two *Nephila* species attacking with thrown silk. Importantly, the thrown silk attack was only employed by naïve juveniles exposed to a novel insect (*Trigona* stingless bees); spiders with prior experience with bees did not use this behavior.

Differences in experience can have an effect on a variety of spider behaviors, from spatial orientation (salticids, Hoefler & Jakob 2006) to mate choice (Hebets 2003). Prior experience alters predatory behavior in some non-web building spiders (i.e., Salticidae, Edwards & Jackson 1994; Skow & Jakob 2005; and Thomisidae, Morse 2000). Although web-building spiders are often assumed to have less capacity to learn, experiments with various species have shown that prior experience affects web architecture (e.g., Heiling & Herberstein 1999; Nakata & Ushimaru 2004; Prokop 2006), propensity to attack particular prey (Herberstein et al. 1998), and searching behavior when captured insects are removed (Rodriguez & Gamboa 2000). Although the conditions under which spiders are stimulated to attack via thrown silk have been studied for several species of Araneidae (Robinson 1975; Robinson & Robinson 1976), there are no published records indicating a role of prior experience in capture tactics used by orb-weaving spiders. Rather, researchers have concluded that the sequence of attack behaviors is a direct response to the prey type and size and the success or failure of a given tactic *during* an attack (Robinson & Mirick 1971; Robinson et al. 1971; Robinson & Robinson 1976; Herberstein et al. 1998; Japyassú & Viera 2002). However, these experiments involved only adult females, and juveniles may exhibit learning as they gain experience with diverse prey types.

Casual observations of capture by small juvenile *Nephila clavipes* (Linnaeus 1767) (Araneae, Nephilidae) indicated that prior experience can influence how a spider attacks a potentially dangerous prey item (Higgins, unpubl. data). In particular, naïve juveniles frequently threw silk to subdue novel large prey items, a tactic previously reported as absent from the prey-capture repertoire of adult *N. clavipes* (Robinson & Mirick 1971; Robinson 1975; Eberhard 1982). To determine the frequency of this tactic in juveniles interacting with novel prey items, I conducted an experiment with laboratory-reared juvenile *N. clavipes* in Panama and casual observations of field-collected *N. pilipes* (Fabricius 1793) in Papua

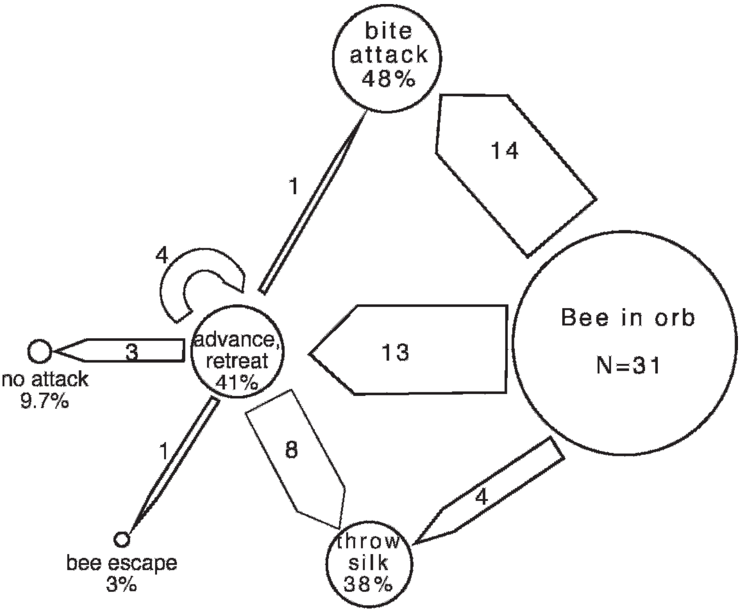

Figure 1.—Behavioral sequences of 31 juvenile *N. clavipes* attacking a *Trigona* bee for the first time. The number in each circle is the percentage of individuals using that behavior (the individuals that repeated "advance, retreat" are only counted once each). The number on each arrow is the number of times that particular transition was observed.

New Guinea (previously *N. maculata,* Kuntner 2005b). Voucher specimens for both species were placed at the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

In December 1985, I collected gravid female *N. clavipes* on Barro Colorado Island, Panama (9°N, 80°W) and housed them in an open-air insectary in the laboratory clearing to collect egg sacs. After the emergence of young and their dispersal to individual orb-webs, I placed groups of spiderlings in screen cages in the insectary, and fed them *ad libitum* with fruit flies collected at banana baits. After molting to the fourth instar, about 0.3 cm leg I tibia + patella, I placed haphazardly-selected individual juveniles on 23–25 cm diameter spherical frames (two intersecting circles of 0.25 cm fiberglass strips) hung in the insectary, upon which they spun orb and barrier webs. Juveniles were offered 3 fruit flies daily (total, about 2.7 mg wet weight) until the next molt, after which they measured approximately 0.5 cm leg I tibia + patella length (TPL).

To determine the prey-capture strategies of these juveniles, I offered each of thirty-one fifth-instar spiders a chilled stingless bee (*Trigona* sp., 3.67 mg mean wet weight, approximately 20% of these spiders' body mass and equal to their TPL). These chilled bees rapidly recovered enough to move, but not to fly, within the time frame of these observations. Occasionally, I over-cooled bees: if dead, I vibrated the bee with a tuning-fork until the spider responded. The experienced spiders readily attacked vibrated dead bees, therefore I interpreted refusal of a dead bee by a naïve spider as rejection of the insect because it was a bee rather than because it was dead. I recorded each individual's response to the bee until the prey was either definitely rejected (after approaching the bee and retreating, the spider remained at the hub > 5 min) or captured and removed to the hub of the web. Chilled bees and dead, vibrated bees are grouped in these analyses due to small sample sizes.

Robinson & Mirick (1971) provide descriptions of adult *Nephila* attack behavior, which I here modify to reflect observed juvenile behaviors. A "bite attack" is an approach followed immediately by a direct bite that is sustained. "Advance and retreat" is an approach followed by retreating to the hub of the web. During a "thrown-silk attack," the spider approached the bee, turned around, and placed silk over the insect using the fourth pair of legs.

The juvenile spiders responded variably to the bees (Fig. 1). The sample size was not sufficient to test for small effects of spider weight or days since molting (age within instar). Of the 27 successful attacks on *Trigona* bees, 15 spiders used the typical *Nephila* long bite attack (one after advance and retreat), followed by pulling the bee from the orb, wrapping it and returning it to the hub suspended from a silk thread. Four spiders immediately attacked the bee using thrown silk followed by a bite, a "wrap-bite" couplet. Many spiders (13) advanced and retreated from the bee. These approaches involved touching the bee, and may have involved attempted bites. Three of these spiders, after one or more approaches, never attacked; one spider approached the bee three times without attacking. On one occasion the bee escaped after the spider had approached and retreated. Of the successful attacks following exploratory approaches, one spider used a bite attack and eight spiders threw silk. Usually, no clear bite was observed until after the spider had removed the wrapped bee to the hub.

I only observed the exploratory approach and subsequent use of thrown silk in naïve spiders. When I repeated the offering of bees to these juveniles, all switched tactics to the long bite attack by the second or, rarely, the third feeding. This behavioral flexibility is distinct from the behavioral sequence plasticity reported for *Nephilengys* by Japyassú & Viera (2002), where mature females altered the attack sequence *during* attacks, but were not *sequentially* tested for shifts in attack strategy.

A thrown-silk attack was seen once in the field in Panama. In this case, a mature female *N. clavipes* (1.2 cm leg I tibia + patella length, approximately 2 cm body length) wrapped but did not bite an approximately 10 mm hemipteran bug. Over a period of 1.5 h, she repeatedly placed silk over the bug using leg IV, capturing it between the thrown silk and the orb-web mesh, and the bug continued struggling and freeing itself. The spider was not observed to bite the bug, and it eventually escaped.

At the Christensen Research Institute (now closed) in Madang, Papua New Guinea (5°13′S, 145°48′E), I recorded attack strategies for sixteen fourth-instar juvenile *N. pilipes* in an insectary during an experiment on developmental plasticity (Higgins 1995). These spiders are the same size as the *N. clavipes* juveniles used in the above-described experiment. All of these individuals were assigned to receive either one or two wild-caught *Trigona* bees daily. I recorded the attack sequence used by these juveniles for the first two bees that were provided. The attack sequences were highly variable, involving six different responses: advance and retreat, touch, long bite, short bite, wrap attack, and cut-silk attack, and were often very long with repeated couplets such as "wrap and bite," "touch and bite," and "touch and wrap." Only 9 (56.25%) of the first attacks were successful; two individuals threw the bee away after subduing it, and five allowed the bee to escape. One individual did not successfully capture a bee until the fourth attempt. *Nephila pilipes* juveniles exhibited one novel attack mode not seen in *N. clavipes*. Eleven (68.75%) of the spiders at some point during their attack cut the orb above or beside the bee so that it collapsed over the insect; two individuals cut the silk as an initial response. Ten (62.5%) of the spiders used thrown silk to attack the prey at some point during the attack (often following short bites or cut-silk attacks). All of the successful attacks included thrown silk at some point of the sequence, and 6 of the successful attacks included cutting the orb. In all cases, attacks on bees after a successful attack were greatly simplified and usually involved just biting, pulling, and returning to the hub (with or without wrapping after removing the bee from the orb).

The exploratory approaches and the thrown-silk and cut-silk attacks are distinct from adult *Nephila* attack behaviors (Robinson & Mirick 1971). "Bite-and-back-off" attacks by adults involve retreating only short distances from the prey, whereas these juveniles retreated to the hub of the orb (16–25 cm radius orb-webs), and often the bee was healthy enough to escape. In addition, the observed thrown-silk attack behavior differs from *in situ* wrapping reported for adult females. Robinson & Mirick (1971) describe *in situ* wrapping by adult female *N. clavipes* as occurring only after the spider has bitten and attempted to pull the prey free of the web; in fact, they experimentally elicited this behavior by restraining prey when the spiders attempted to pull it free. The juveniles I observed were capable of pulling the bee free and wrapping; in *N. clavipes*, all direct bite attacks were followed by this behavioral sequence. None of the juveniles that threw silk attempted to pull the bee free before using this tactic.

The thrown-silk attack by *Nephila* greatly resembles the wrap-attack of araneoid spiders. After approaching, the *Nephila* juveniles turned away from the prey and threw silk using legs IV. Unlike wrap-attacks, the bees did not rotate but became sandwiched between the silk mat thrown by the spider and the fine mesh of the orb. The spider would then cut the bee free of the orb, attach a silk thread, and carry it to the hub hanging from this thread. Biting followed the return to the hub. The main difference between the *Nephila* thrown-silk attack and the araneoid attack wrap is the failure of *Nephila* juveniles to rotate the prey "rotisserie-style" around a radius or several viscid spiral strands between two radii (Eberhard 1967). This difference may reflect physical constraints imposed by the extremely fine mesh of the juvenile *Nephila* orb web. With a body length of 0.4–0.6 cm, *Trigona* bees are larger than the 0.1 cm orb mesh of *Nephila* juveniles (Higgins & Buskirk 1992).

It is widely reported that *Nephila* spiders lack the "attack-wrap" or "wrap and bite" behavior that other orb-web spiders use to deal with large or potentially dangerous prey (Robinson & Mirick 1971; Robinson et al. 1971; Robinson 1975; Eberhard 1982; Kuntner 2005b, 2006). Most recent studies have focused on adult females, to standardize the assessment of this character for phylogenetic analyses (Kuntner pers. com.). The use of a thrown-silk attack by juvenile *N. clavipes* and *N. pilipes* is correlated with each individual's experience with these relatively large insects. *Trigona* bees are potentially dangerous prey for small *N. clavipes* and I have observed them kill juveniles in Panama. The observation of an adult female *N. clavipes* using this attack against a bug, perhaps chemically defended, indicates that the behavior is not lost with maturation. It is possible that the rarity of the thrown-silk attack is due to the large size of mature females and the relatively small size of the common prey items (Rypstra 1981; Nentwig 1985; Higgins 1987; Higgins & Buskirk 1991). Future studies should address why this tactic is so rarely employed and whether the observed differences with the classic wrap-attack behavior simply reflect a physical constraint due to the fine structure of the *Nephila* orb web.

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