

**Study on the Chemical Communication between the Microhylid Frog,
Chiasmocleis ventrimaculatata, and a Theraphosid Spider involved in a
Commensal Relationship**

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Introduction

Microhylid frogs are small, ground-dwelling frogs known to specialize on small insects such as ants and termites as prey. A relationship exists between certain microhylid frogs and tarantula spiders (theraphosids). The small frogs of several genera live in the burrows of theraphosid spiders that normally prey on animals of their stature. It is known that the two organisms will share the same daytime retreat, the spider's burrow, and come out to forage at night. Adult female spiders aggressively prevent other large organisms from entering the burrow but show no reaction to the frogs. Microhylid frogs are within the size range of animals normally eaten by the spider. However, they are tolerated in and around burrows and on occasion frogs will even take refuge under a spider in response to a disturbance. The frogs are reported to be non-territorial, and several individuals may inhabit the same burrow.

Between September 9 and December 12, 2000 I observed the frog *Chiasmocleis ventrimaculata* and its burrow mate, a theraphosid spider whose species I am unsure of. I attempted to show that a chemical signal in the frog's skin is used by the spider to identify the frog and reject it as prey.

Background

Why the spiders do not prey on their microhylid guests is still unclear. The frogs definitely fall within the range of prey sizes normally taken by the spider. The fact that young spiders have been observed to pounce on frogs and only after examining them under their chelicerae release the frog unharmed would indicate some, as of yet unknown, communication stops them. Previous studies implicate the use of chemical cues used by the spider to recognize the frog (Foelix 1982, Crocroft and Hambler 1989).

The possibility does exist that these frogs are poisonous or at least unpalatable to their burrow mates. Powell et al. (1984) studied an association of the leptodactylid frog *Physalamus pustulosus* and theraphosids of *Aphonopelma* genus in Mexico. His research suggests that noxious skin secretions protect *Physalamus pustulosus* from being eaten by the spider *Aphonopelma*. *Physalamus pustulosus* is preyed upon by a wide range of vertebrate predators leading Powell (1984) to suggest that the frog's apparent immunity to predation by *Aphonopelma* may indicate a chemical defense that evolved in response to invertebrate predators. Hambler and Crocroft believe this may also be the situation for *Chiasmocleis ventrimaculata* but an analysis of skin toxicity has never been done.

Toxicity studies have been performed on a similar microhylid species *Gastrophryne carolinensis*. This species also has been reported to be a cohabitant of tarantula burrows (Blair 1936). Garton and Mushinsky (1965) found potential vertebrate predators ate this microhylid significantly less than control anurans, and ingestion of them was often followed by prolonged periods of distress by predators. They also found that injections of 3 mg of *G. carolinensis* skin per gram body weight killed mice within 15 min while that of the control *Rana catsebieana* caused no observable distress behaviors. Several species of snake are known to prey on *Gastrophryne* species as well as shrews which were reported to consume *Gastrophryne olivacea* but only after removing and discarding the skin and feet (Anderson 1954).

The behavior and gland characteristics of these small nondescript microhylids have lead Garton and Mushinsky (1965) to postulate that their noxious skin secretions evolved as a defense from counter attacks of their preferred prey, ants. They theorize that, once established, the same mechanism could be a defense against predators as well as prey. Support for this was found through the use of scanning electron micrographs that show the poison glands of *Gastrophryne carolinensis* are spread over the entire skin surface (extending down the limbs to the palms of the hands and feet). This is different from most toxic amphibians where the poison glands are concentrated in areas predators are most likely to contact first and accompanied by bright warning coloration. Garton and Mushinsky suggest that the even distribution of poison glands is more effective than patchily arrayed glands as a defense against the swarming attacks of ants. If so, they further postulate that while vertebrate predators may be able to consume a frog with only some ill side affects, invertebrate predators may be more sensitive to the toxins of these microhylids. Typical defense posturing of poisonous amphibians has never been observed from *Gastrophryne* species. Instead *Gastrophryne carolinensis*, like nontoxic *Rana* species, remains immobile in response to a potential predator. *Gastrophryne carolinensis* has been found in the nest of fire ants (as well as other ant species with less painful bites) and appear to be immune to their prey's normally painfully effective self-defense (Garton and Mushinsky 1965, Anderson 1954). Swarming ants are known to become entangled in the skin secretions of *Gastrophryne carolinensis*, the frog can later remove the ants, many of which are dead, and the secretions by rubbing against the substrate (Anderson 1954).

The three known associations of frogs and theraphosids do not appear to be obligate to either frog or spider. The proportions of the populations involved are unknown in all examples. Approximately 75% of over 100 burrows examined by Blair (1936) contained at least one *Gastrophryne olivacea*, and during the driest weather this was apparently the only microhabitat where the frogs could be found. Conant (1975) reported *Gastrophryne olivacea* to be found in spider burrows as well as a range of other microhabitats. Crocrot and Hambler collected *Chiasmocleis ventrimaculata* from the forest litter and low herbaceous growth away from burrows, and it is reported to breed in flooded areas of the forest (Duellman 1978, Crocrot and Hambler 1989). They did not document the proportion of *Xenesthis immanis* burrows without frogs.

It seems obvious that for the frogs, a spider burrow will provide a favorable microhabitat. By preventing other animals such as snakes from entering the burrow, the spider inadvertently is preventing potential frog predators as well. Crocrot and Hambler (1989) also suggest that the proximity of discarded spider prey parts may result in a local concentration of invertebrate food items. Powell et al (1984) propose that inhabiting a spider burrow may reduce the risk of desiccation for a frog. What, if anything, the spider gains from this relationship is far less obvious. Hunt (1980) speculates that ants are potential spider egg predators and that ant-specialist microhylids, by reducing the number of ants around the burrow may reduce predation on the spider's eggs.

The Spider

Crocrot and Hambler had performed their study in the same region of Peru that I worked and identified the animals in the relationship as *Chiasmocleis ventrimaculata* and *Xenesthis immanis*. However, *X. immanis* is not known to occur in Peru, and the spider I found *C. ventrimaculata* living with does not fit the description of *X. immanis*. *X. immanis* is easily identified by the bright pink on its carapace. This characteristic was never noted on any of the spiders I observed. It appears that *C. ventrimaculata* may even live with two different species of theraphosid spider. One that was heavy bodied and solid black, and the other less robust with red hairs on the abdomen. I did not attempt to properly key out the spiders and this should have been done. It is the opinion of an arachnologist, Martin Nicholas who I have been in contact with that the solid black spider is a member of the genus *Pamphobeteus*, it has not been described as a species and is known to the locals as the chicken spider due to an anecdotal tale of it carrying off a chick.

Search Methods

On September 9, 2000 my field tech and I began walking the trails at Posadas Amazonas Rainforest lodge in search for the burrows of the Chicken Spider and the frog *Chiasmocleis ventrimaculata* who live together in a commensal relationship. I decided it would be best to first systematically search all the trails because all previous research and anecdotal literature claimed that the spider preferred to make her burrows at the edge of trails or man-made clearings. We started out each night at 19:45, just after dinner. Each of us wore a headlamp, and one of us would look left, the other right for approximately 3 meters off the trail, scanning the ground for either member of the commensal relationship. We paced slowly, treating the trail as a continuous transect.

Burrow 2: On September 14, at 21:15 we found the first burrow on Trail K. One adult female was positioned just outside the entrance, which was located under a rotted stump, and five juvenile spiders were seen near her, three on the ground and two on the stump. The furthest separated spiders were approximately 50 centimeters apart. Closer inspection of the stump revealed 2 smaller entrances on either side of the main entrance. The juveniles on the stump were each positioned above one of these entrances and scurried inside them when disturbed. Three *C. ventrimaculata* were spotted within two meters of the burrow. The burrow was marked with pink flagging. That night we continued to search Trail K and did not find another burrow. On the following night, September 15, we returned to the burrow and found five spiders sitting within one meter of the burrow. The adult female sat 10 centimeters in front of the burrow entrance. Four juvenile spiders were seen outside the burrow. We captured these spiders, recorded their distance from the burrow, measured and marked them and then placed them in a 10 gallon bucket for holding (see table 1). When one spider was captured the disturbance would scare the others back into the burrow. After between 5 and 10 minutes the spiders would re-emerge. There is no way to be sure that the spiders who re-emerged were the same ones that had been previously out, but as no more than four, the number originally seen out, were captured without coaxing out of the burrow, this seems intuitive. Each spider was marked with a unique code using blue and/or yellow enamel paint and a cotton swab. A dot of one color was placed on a leg. The first spiders were marked with just one dot, then combinations of colored dots were used. After the four spiders originally seen outside the burrow were captured and processed, we

peered inside the entrance and saw there were other juveniles inside the burrow. A cricket was taped to a stick and used to 'fish' the juvenile spiders out. An additional 11 spiders were captured with this method and processed. One spider was only seen and could not be captured, it started to chase the cricket on a stick but ran back inside and refused to chase again. Using flashlights to look inside the burrow we could not see any more spiders. However we cannot be sure all were noted because only a small portion of the burrow was visible and even the spider that got away could not be seen. We did not see any *C. ventrimaculata* again at this burrow until October 11, despite thorough searching on several occasions. Only one was ever captured and marked at this burrow.

Burrow 9: On September 17, an adult female spider was spotted outside her burrow on the trail leading to the workers river port at 15:30. One juvenile spider was near her and another could be seen just inside the burrow entrance. They could not be captured. Approximate sizes of the adult female and juvenile are 70 millimeters and 25 millimeters respectively. We never saw *C. ventrimaculata* at this burrow.

Burrow 5: The next burrow was found on September 20 on Trail H. One adult female was seen with 10 babies approximately 20 millimeters or less. Because these juveniles were so small, they were very difficult to capture. Plus, it was difficult to handle these tiny spiders without causing them injury so we did not mark and measure them. The burrow was under a large root of a dead tree, the root still attached to the ground. One large adult female spider had been seen three meters from the burrow before it was sighted in the roots of a fallen tree 4.5 meters from the ground. She was not captured and was not seen again.

After all the trails had been used as transects we began doing quadrats in the forest. Between 10 and 17 quadrats were done on each trail, at regular intervals depending on the length of each trail. We moved 2 m away from the trail in a randomly chosen direction and blocked off a 5 x 5 m square. Two people then searched this square. One spider was found on October 2 alone in a quadrat done on trail D. It was marked and measured and the area surrounding the quadrat was searched for approximately 3 meters in each direction but no burrow was found.

On September 29 an adult female was spotted outside of her burrow while walking on the Tower Trail between quadrats. No juvenile spiders or *C. ventrimaculata* were ever seen with her.

Burrow 3: On October 3, a burrow was found off of trail G inside a quadrat. The burrow was 6.7 m from the trail and had two entrances 55 centimeters apart. One adult female spider was outside of the burrow and five *C. ventrimaculata* were within 1 m of the burrow. On October 4, two adult females were captured at this burrow. One measured 60.4 millimeters and was marked YR1YL1. The other measured 73.5 millimeters and was marked BR1BL1. Nine *C. ventrimaculata* were captured and marked.

Burrow 4: Another burrow was then found approximately fifteen meters from this along Trail G, 1.2 meters from the trail. It was found while walking along the trail between quadrats. A small amount of webbing was seen at the entrance, which is not something this spider is known to do. Webbing was not seen at any other burrow. One adult female was captured, measured 62.6 mm, and marked YR2YL2. No juveniles were ever seen there. Fifty centimeters from her burrow, there was what looked like another entrance to the burrow. However, closer inspection revealed that this was a short tunnel where a wolf spider was seen most nights. On two occasions an individual *C. ventrimaculata* was observed but one was never captured at this burrow.

On October 6, one *C. ventrimaculata* was seen in a quadrat on Trail F but it could not be captured. On October 9 one *C. ventrimaculata* was seen in a quadrat on Trail H, which also could not be captured. No burrow was found in either quadrat or their surrounding areas.

Burrow 6: On October 2, a spider was seen standing in the middle of Trail H leading to the discovery of Burrow 6. This burrow appeared to be one family with 3 widely separated holes. Hole 1 was 4.5 meters from the trail, Hole 2 was 2.1 meters from trail and 1.9 meters from the Hole 1, Hole 3 was 5.1 m from Hole 2 and on other side of trail 1.8 meters from Holes 1 and 2. An adult female spider was seen outside of the first hole. She was never seen outside of any other hole. She could not be captured and was approximately 70 millimeters. More than 20 juvenile spiders were seen at a given time. These juveniles varied in size more so than any other group of juveniles at a single burrow. They ranged from approximately 25 – 50 millimeters. Twenty *C. ventrimaculata* were captured and marked at this burrow.

Burrow Excavation

On October 17 Burrow 5 was excavated using a small spade. This burrow ran the length of a root of a dead tree. The burrow entrance was 21 centimeters at the base, its widest point, and became narrower toward the top. There were numerous small holes along the root which the juvenile spiders and frogs were observed to use. The burrow was approximately 10 centimeters in diameter and 110 centimeters long and ran along the length of the root. Four juvenile spiders were found in the first 50 centimeters starting from the entrance. There were twenty-two juvenile spiders found in total, all but the four in the furthest rear portion of the burrow with the adult female. Spider BR3, which had been removed from Burrow 2 and placed there during an experiment, was one of these twenty-two spiders and was found towards the end of the tunnel. The burrow was slightly wider towards the end but there was not a well-developed chamber. The female and juveniles were merely dispersed in the last 20 centimeters of the burrow. No *Chiasmocleis ventrimaculata* were at first seen during the excavation but the root was decomposed with many crevices for frogs to hide in. During closer inspection, after the excavation, three frogs were found in the first 20 – 30 centimeters of the burrow. An old egg sac was also found right near the burrow containing many dead spiderlings. Because the juvenile spiders were difficult to handle without injuring them, only what was judged to be the smallest and largest were measured. These measured 20.5 centimeters and 12.8 centimeters respectively. The adult female was 73.2 centimeters and BR3 was 38.1 centimeters. Spiders were stored in a 10 gallon bucket and taken to a nearby area and released in what looked like an unused burrow. The spiders ran inside this hole, but they were never seen near it again. That evening 10 juvenile spiders and one *C. ventrimaculata* were seen near the destroyed burrow. Juvenile spiders and *C. ventrimaculata* were seen along the destroyed burrow until December when the observer left. Seven *C. ventrimaculata* were captured and marked at this place between October 17 and December 11, 2000.

Experiments

Experiments were done with seven species of ground dwelling frogs to see which species the spiders normally eat. Because these spiders are burrowers and could be assumed not to run into a tree frog with any frequency, only ground dwellers were tested. Frogs were captured when the opportunity arose, and then offered to a spider by gently placing the frog 2 – 5 centimeters in front of a spider. Individual frogs that were not eaten by a spider would be tested again with a different individual spider (Table 2). To be sure the spider was hungry it was always offered an insect, it was known to eat, as a control.

A set of experiments was done to discover if it is a chemical in the skin that prevents the spider from eating *C. ventrimaculata*. The first used a whole skin placed on another species of frog and was considered successful. The others attempted to extract the signal chemical from the skin by mixing it with various solvents.

One *C. ventrimaculata* (21.7 mm) was killed by placing the frog in a small plastic container with a cotton ball soaked in alcohol. When the animal was dead, its skin was removed and placed on an *A. andreaei*. The skin was pulled off of *C. ventrimaculata* and then placed onto *A. andreaei* (38.3mm), putting the frogs front legs through the 'sleeves' of the *C. ventrimaculata* skin to secure it. The skin was then adjusted to cover as much of the *A. andreaei* as possible. Then, this frog in its *C. ventrimaculata* 'coat' was gently placed 2 – 5 centimeters in front of a spider. This experiment was done 5 times with a different individual spider using the same *A. andreaei* in the same 'coat.' The first four times the frog was captured by the spider, held under its pedipalps, examined for several seconds and then released. To be sure the spider was hungry it was always offered a cockroach, which it was known to eat, as a control. The fifth time the frog was held and examined and then the spider took the frog inside its burrow. After one hour, the frog was presumed eaten and the observers left. As time had passed, the skin 'coat' had become less supple and it covered its wearer less and less, who was larger than the coat's original wearer. It is possible that the last spider was able to contact enough of the *A. andreaei*'s true skin that it could decipher it as normally eaten prey. It is also possible that chemical signal in *C. ventrimaculata*'s skin was volatile, and no longer part of a living animal, no more of the signal chemical was produced, and it had evaporated away over the course of the evening (Table 3). From this experiment it was concluded that a chemical signal probably does exist in *C. ventrimaculata*'s skin. However, it needs to be repeated to increase the sample size in order to perform a statistically significant Chi Square Test.

The next series of experiments used various chemical solvents mixed with the skin of dead *C. ventrimaculata*. This was done to get a better idea of the chemicals potentially used by the spider to identify *C. ventrimaculata* and have a medium easier to work with than a skin 'coat'.

In the first experiment water was used as a solvent. One live *C. ventrimaculata* was placed in 30 ml of water and allowed to soak in it for 24 hours. Another frog was then placed in this water, with the *C. ventrimaculata* still in it for 5 hours. *Adenomera andreaei* was chosen because of its abundance, and it had been found in a previous study (Crocroft and Hambler 1989) that the

spiders would eat this frog. This treated *A. andreae* was placed in front of spider YL4 who captured it but did not eat it. It was impossible to tell if the spider had released the frog, or it had escaped. Then, YR4 captured and ate the frog. It was concluded that the chemical signal was not soluble in water.

Second, solvents were tested as controls to be sure it was not the solvent that stopped the spider from choosing the animal as prey. Ethanol, hexane, methanol, acetone, and TRIS were swabbed onto cockroaches or lightning bugs (both had been observed to be eaten by the spider). The insects swabbed with hexane and acetone were not eaten and the animal swabbed with hexane died soon after. The other solvents, ethanol, methanol, and TRIS did not deter the spiders from eating. The skin of *C. ventrimaculata* was then removed from two frogs. The frogs were first killed by placing them in a small plastic container with a cotton ball soaked in ethanol. Each skin was cut up with a scalpel and put into 2 ml of ethanol or methanol. The solutions were allowed to sit for one hour and then were swabbed onto cockroaches. The cockroaches were placed in front of spiders that promptly ate them. After two trials, the skins were removed from the solvent and ground between two rocks. This material was then put back in the solvent; the solution was shaken up and then allowed to sit for five minutes. Each was then swabbed onto a cockroach and offered to a spider. The roaches swabbed with the ethanol solution were eaten. Those swabbed in methanol were rejected. To be sure the spider was hungry, it was always offered a plain cockroach as a control after it refused to eat an experimental cockroach (Table 4). These cockroaches should have been swabbed in methanol to make a more accurate control where only the presence of chemicals in the frog skin could account for the difference in behavior. However, those first experiments before the frog skin was ground could be considered complete controls since the insects were covered in methanol that apparently did not contain the chemicals in the frog skin.

One experiment was done with what was believed to be an adult male theraphosid of the species living with *C. ventrimaculata*. A frog was placed in a bucket with this male theraphosid. It was eaten. It was known that the spider had been in captivity some time and not fed. Unfortunately, the spider escaped so the experiment could not be repeated. It would be interesting to see if other species of theraphosid found in this region will also reject *C. ventrimaculata* as prey. Single experiments were also done with a wolf spider and an orb-weaving spider. The wolf spider did capture *C. ventrimaculata* when it was first placed in the bucket. However, it would not eat the frog even when left with it for 48 hours in a small bucket. It did eat 3 cockroaches during this

time. This experiment needs to be repeated for statistical significance. On October 12, one *C. ventrimaculata* was placed in the web of an orb-weaving spider. The spider killed the frog and appeared to be feeding and/or killing the frog for 50 minutes. It was then dropped from the web. Whether this was an accident or purposeful action was impossible to note so I do not see any value in repeating this experiment.

Conclusions

This study indicates that the spider and the frog *C. ventrimaculata* communicate via a chemical signal. However, because the proper controls were not done, the theory cannot be supported statistically. I would like to return to Posadas Amazonas to perform these controls. If possible I would also like to collect 12 live *C. ventrimaculata* and 12 spiders. This would be to analyze the frog skins to isolate and identify the signal chemical. In addition the spider specimens could be used to name this undescribed species.

Mother/Juvenile Spider Identification Experiments

During my study I found adult female spiders living in burrows with and without young. Knowing the cannibalistic nature of most spiders, I was curious to understand how the mothers recognize their offspring to reject them as prey. I transplanted babies between adult female spiders. I found that mothers with babies will accept new babies, and the introduced juvenile will continue to reside at that burrow. Mothers without babies killed and ate juvenile spiders that were placed near them (Table 6). It appears that these spiders rely on vibration detection as a primary sense. I also noted that when the same species of methanol extract treated insect was offered to a spider, more than 3 times in a row, that spider would no longer attack the insect. It may be interesting to carry this experiment out in a more systematic way to understand how these spiders learn vibrations and use them as behavioral cues.

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