Final Report Colletidae Research

Chrisitne Dimke

May 2016

1 Overview

Over the course of this year nest specimens from four of the five Colletidae subfamilies and annalyzed their properties. It was demonstrated by previous work at this institution that the nest cells of at least one specie of Colletidae were not in fact just layers of polyester, but a composite of polyester and silk. The aforementioned work culminated in a Masters thesis at the University of Bath by Rebecca Belisle (Belisle 2011). The focus of this research was to compare nest cell characteristics with what we know about the nesting behavior of the bees across all subfamilies, the documentation of which is extensive. It was found that the nesting behavior of each subfamily heavily correlated to the cell characteristics observed under our testing.

2 Material Science Background

In 1979, in Science, Hefetz published the first non-entomological study of solitary bee nest cells. The study dealt with examples of nest cells from three different *Colletes* species. After finding the cells to be resistant to chemical degradation they were treated with chloroform and Mass Spectrometry was used to analyze the upper hydrophobic layer of the resulting solution(Hefetz, 1979). The study determined that the nests were comprised of layers of polyester and the material was dubbed a laminester in order to indicate is layered nature. After that time, however, the nests were forgotten about and the next work published about them was that of Belisle in 2011. Her thesis published in depth analysis of two *Colletes* species *ineaqualis* , native to the northeastern United States, and *halophilus* a resident of England. In her preliminary research at Olin College it was discovered that there were fibers on the outside of the nests were crisscrossed with fibers. Subsequent tests run on the fibers have been consistent with silk. Particularly important were Amino Acid Analysis carried out on the cells as well as protein specific staining and imaging of both the inside and our side of cells(Belisle, 2011). This study analyzes different species and subfamilies in the Colletidae family.

3 Hymenoptera Adult Silk Production

It is uncommon in the literature to see references to silk spinning not done by Larva or pupa during their development. While the spinning of cocoons certainly happens and is important both for reproduction and commercial means it is not the only instance. Many adult Anthropoids also spin, the most common example being that of Arachnids. Arachnids (which are not closely related to Hymenoptera) aside, adult insect spinning is rare, and was almost unheard of until the 1990's. In the Hymenoptera order it has been reported in 8 genera and among those one, Colletidae, has only one genus currently represented, *Hylaeus.* (Fischer and Robertson, 1998).

Silk is usually divided up into two to three main groups $\alpha - helical, cross - \beta, and parallel - \beta. Each is primarily made up of gly$ $helical is a helix as one might expect and owes its shape to the hydrogen bonds that form between the amine and carboxyl groups. This ty$ $Zacarin, Silva De Moraes, and Taboga, 2003) and also the colleterial glands (Craig, 1997), and this is the most studied family.$

There is little and often contradictory and incomplete data on adult Hymenoptera spinning habits, but has been shown that *Colletes halophilus* adults do produce a silk material. Although it seems that some authors thinks this precludes the production of polyester (Torchio, 1984) and that the only secretions are laid down by the bee, newer research would indicate that is not the case. The observations by such authors may be incomplete because they state that the secretions come exclusively from the mandibular glands, but also report viewing the bees turning around with frequency at the top of the tunnel which would give it the opportunity to ingest temporarily the secretion of the Dufour's gland into the crop. Observations of Hymenoptera adult silk production in non-Arachnids is relatively recent and the has lead to closer inspection of previously documented materials produced by many in this order.

4 Entomology and Nesting Biology

Unlike material characterizations the analysis of nests and nesting behavior of members of the Colletidae family is extensive. In the Colletidae family there are approximately 2500 species of Colletidae on every content except Antarctica (Almeida, 2009) and each subfamily has adapted certain ways of dealing with different environments. The five subfamilies *Colletinae*, *Hylaeinae*, *Ptilogossa*,*Xeromessilinae*, and *Euryglossinae* each with defining characteristics.

The superfamily Apoidae or Sphecoidae (antiquated) contains both Bees and wasps, and although there is an ongoing discussion on how to organize the group into families it is clear that within each there are one or more families of bees (Ampiformes) and likewise for wasps (Spheciformes). FigureXX shows Michener's classification which is the most commonly accepted (Michener, 2000). Pertinent characteristics of this group include nesting patterns, wing shape, and other anatomical similarities. It is believed that Bees are a decedent of an ancestor we would classify as a Speciform today although this does not make the superfamily Monophyletic because Speciforms are incredibly diverse; although it is likely that the group is Paraphyletic or more closely related.

Colletidae is a member of the Short-Tongue Clade of bees. A group that is paraphyletically related to the Long-Tongued clade. Colletidae also encompasses all the bees traditionally thought of as "lactone producing". It is a diverse family and some have proposed splitting it into several, but all Collitid species share common glossal features unique to Colletidae. They are noted for having a bilobed tongue which along with the family's distinctive hairs present on the glossa allow them to paint on a DuFour's gland excretion which hardens into a "cellophane-like" plastic used to construct individual nest cells within a series of tunnels usually excavated out of clay like soil, although there are some species which nest in preexisting holes in rock or soil (often from former nests of Colletidae or other members of Hymenoptera) or in "pithy stems" . The characteristics of the glossa in Colletidae are what set them up to be considered a sister to all other bees. These features are enough to justify using the term monophyletic for this family and also to prevent splitting for now.

Bees can have more or less hair, but all bees have at least some. This linkage is important and helps distinguish Colletidae, one of whose most consistent features are their glossal hairs, as the primary candidate for containing the proto-bee more specifically the genus *Colletes* is noted as being one of the most primitive families indicating the proto-bee would have been part of this group or at least very closely related.

The characteristic of Colletidae's particularly evolved hairs are listed as the first piece of proof for the group's monophyletic status (Michener, 2000) the argument begins as follows:

"Character a. Some of the hairs of bees are plumose or at least branched. In most Hymenoptera they are simple, although plumose hairs are found in some other groups, e.g., some Mutillidae. Contrary to the usual opinion I doubt that plumose hairs of bees arose as pollen-collecting and pollen-carrying structures, although of course some bees take advantage of plumosity to enhance these functions. In many bees the scopal are simple, yet nevertheless carry pollen, showing that plumosity is not necessary for a pollen carrier. Moreover, plumose hairs are often found in locations where pollen is never carried, e.g., around the anterior thoracic spiracles and on the male genitalia and hidden sterna. Further, hairs are branches (plumose) in many different ways, some of them not at all suitable for pollen-collecting or pollen-carrying, indicating that the degree or type of plumosity may be under carious selective pressures having nothing to do with pollen.

Possible plumosity first arose as one way (an alternative to a great number of hairs) in which forms in a xeric environment could decrease air flow near the integumental surface, and thus reduce water loss. Simultaneously, since hairs are often pale, plumosity could have been one way to increase the pale coronation often characteristic of insects in xeric environments. Presumably pale coloration both reflects heat, helping to prevent overheating, and serves for protective coloration on the pale soils and pale vegetation characteristic of deserts."

-Michener, The World of Bees, p. 60

All Bees have two glands near their sting which in a time close to their discovery were referred to as the acid and basic glands(Cane,1983), the name was an excellent indicator of their contents, but did little to illuminate their function. Although early on some thought that both the acid and basic glands contributed to the venom it has been fairly clearly shown that venom comes solely from the acid gland which has led to its new name, the venom gland . The basic gland remained a mystery for a number of years after it was recognized, early thoughts that emerged after the realization that it did not contribute to the venom hypothesized that the excretions were spread over the eggs to protect them. Although not accurate in terms of execution general feelings were proved correct. Now called the Dufour's gland it is recognized to excrete a lactone containing substance that when applied by Collitid with their tongue (for diagrams refer to Michener, 2000, p. 83-85) polymerizes and forms the "cellophane-like" substance that they use to create their nest cells. Although all bees have both glands they are often differentially developed. In Colletes for example the Dufour's gland is very well developed representing the majority of the abdominal cavity, while the acid gland is small and under developed . This is consistent with the general statement that Colletidae don't sting, although they have stings and some venom, they do not use it unless handled extensively.

All Collitids are solitary, although this does not preclude aggregations which have been known to happen. Of the Colletidae that nest in the ground, which tend to be the species/ genera with more hair, the structure of nests seems to be fairly uniform. As seen in the figure to the left it is a main tunnel at a near right angle to the ground above, with side tunnels each ending in one nest cell which are often back filled when they are completed. Possibly contrary to intuition there appears to be no correlation between the depth of the cells and the order of their creation (Rozen, 1984).

It is also worth noting that with few exceptions the provisions of Colletidae are liquid as opposed to being a ball of more or less (sometimes moistened with nectar) packed pollen in other solitary species. This could be a reason for the development of the polymer based nest cell linings as they form a watertight airtight barrier which the nectar cannot permeate. The larvae do not spin cocoons (with the exception of Diphaglossinae whose placement in this family has been questioned), and it is this family that is known for making nest through the two step polymerization method .

The fact that Colletidae and more specifically Colletes make nest cells out of a "cellophane-like" material has been well known for some time and has been commented on in the literature frequently as an identifying characteristic, but it wasn't until 1979 that the chemical structure of the linings was examined and correctly identified as being, at least in part, a product of the Dufour's gland . The lining has been shown to be water proof as well as an effective barrier to bacterial and fungal presences . It remains unknown exactly how the long chain lactones are produced, but most assume that somehow the Salivary or Mandibular glands are involved. When analyzed secretions directly from the Dufour's gland showed the presence of many lactones, but all in short chains and when the samples were allowed to dry on a glass plate they did not harden into the classic cellophane-like material the nests are noted for (Espilie, Cane, Himmelsbach, 1992).

4.1 *Colletinae*

A widespread family present on every inhabited continent except Australia. Much of what is known about the *Colletinae* is gleaned from studying the *Colletes* genus. Nesting in sandy soil they dig strait tunnels straight down into the ground with horizontal side tunnels where the nests are laid. Although their nesting behavior is remarkably uniform *Colletes* species can be found in a variety of climates. Their family patterns largely follow those described by Batra in a paper specifically concerning *Colletes inaequalis*, *C. thoracicius*, and *C. validus* (Batra, 1980).

4.2 *Diphaglossinae*

The only subfamily to still spin cocoons is the Diphaglossinae are found in the Americas as far north as Texas and Arizona. They are the largest of all Colletidae and a proprietary ground nest digging group, the latter being consistent with the family as a whole. Bees of this subfamily are typically large in body and solitary although aggregations are present as with most Colletids (Rozen, 1984). Once tunnels are constructed the

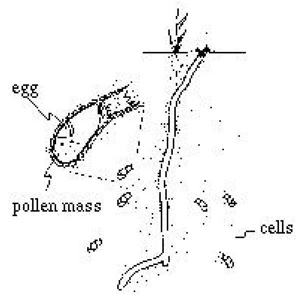

Figure 1: Side View of typical *Colletes* nest network

bee will create the nest cell, provision it with the usual pollen and nectar, but also an oily substance that is presumably also of floral origin, the lid to the cell is not made at the same time as the lining and secured afterward as is the case with other Colletids, but instead is made after provisioning is complete and attached loosely if at all to the cell lining. This allows a cotton like "wafer" of what is suspected to be mold to grow between the egg and subsequent instars and the cell closure. This is the only subfamily within Colletidae noted for having larvae that spin cocoons.

4.3 *Euroglossinae*

This Subfamily is endemic to Australia and nearly exclusively so, there have been a handful of sightings in South Africa and there is one established specie in New Zealand, this species exists in both New Zealand and Australia indicating its fairly recent migration (Donovan B. , 1983). They are small, some even minute bees that do not have the metallic coloring characteristic of some genera of Colletids and have relatively broad faces. (Michener C. D., 2000). Euryglossinae do not have a predisposition to digging their own nests and instead prefer to take up residence in preexisting holes in wood, presumably leftover from beetle activity.

4.4 *Xeromessilinae*

A lesser described yet widespread primarily South American subfamily, Xeromelissinae share an apparent lack of scopa, small stature, and nonmetallic appearance with Euryglossinae. This is not to say that there are no hairs, but rather that they are sparse and lack the plumose nature which is associated with the ability to carry pollen on the hind legs, but specimens show that the pollen is effectively carried on the hind legs as in Colletidae with more scopal hairs. (Michener C. , 1995) They share nesting characteristics with the genus Hylaeus nesting in wood or stems and creating linearly sequential cells with the cellophane-like lining characteristic of the family (Michener C. D., 2000).

5 Methods

Nests were gathered from five species and four subfamilies namely *Ptiloglossa arizonesis*, *Hylaeus basalis*, *Xeromessilinae spp.*, *Colletes inaequalis*, and *C. spp.*. Many of the specimens had been kept in lab conditions since the 1980s leading to the disappearance of plasticizers and leaving them brittle. *Colletes ineaqualis* was obtained through direct collection at a site in central MA in May 2015, Xeromessilinae were acquired from the University of Toronto, and all others are from Jerry Rozen at the American Museum of Natural History. Once received specimens 4 degrees Celsius.

Specimens were separated from the rest of samples by first cutting away as much of the surrounding material as possible followed by repeated soaking in Ethanol(if necessary Isopropyl Alcohol) and drying overnight at 45 degrees Celsius unitl complete seperation was achieved. Upon separation nests were sectioned, sputter coated with Ag, and imaged in a Joel JSM-6060LV SEM both on the inside and on the outside.

Other sections were stained using SYPRO Ruby a protein specific fluorescent stain. They were immersed for 96 hours in the stain and rinsed afterwords with ethanol. They were then imaged in an Olympus

Fluorescent microscope and. After imaging the stained samples were sent to the university of California Davis for Amino Acid Analysis.

6 Results

Not all test results have so far been collected.

6.1 SEM

For three of four subfamilies were found to have fibers on the outside of the nest cell. The *Ptiloglossa spp.* had a smooth outer surface which is consistent with known nesting ecology. It has been noted in more recent literature that Diphaglossinae have thinner nest linings and lack any fibrous material. Although their are contradictory reports on subfamily trends they are widely accepted as distinct from other subfamilies in larval activity as well as structure of nest (Rozen, 1984). Imprints of a textile pressed to the lining from the inside were observed and are believed to have been placed there due to extended exposure to a larval cocoon. All other samples were observed to have fibers on the outside and a smooth inside consistent with *C. halophilus*.

6.2 Flourescent Microscopy

Tod date only *C. inaequalis* has been imaged, but the outside of the stained cell showed fluorescent fibers on a dark background. Similar results were reported for *C. halophilus*.

6.3 Amino Acid Analysis

Among the species tested, *C. inaequalis* and *C. halophilus*, the latter having been done by Belisle, results have been consistent with α- helical silk. This is indicated by the presence of alanine and serine. Results for *C. halophilus* can be found in the figure below.

Figure 2: Combustion Analysis as Reported by Belisle

Element	Run 1 (wt %)	Run 2 (wt %)
Carbon	59.22	59.06
Hydrogen	9.60	9.46
Nitrogen	1.54	1.55
Oxygen	15.76	15.61
Sulphur	0.0	0.0

7 Discussion and Current Work

All together the preliminary results from this study indicate that for at least three of the subfamilies produce silk-polyester composite. It is important to not that there has been no identified origin of the polymerization agent nor of the silk precursor. The indication here of the α-helical structure would cause reason to believe that certain glands may be implicated in its production. This silk production by the adult bee is distinct from that known of the Hymenoptera previously which consisted of cocoon spinning by larvae and may be more closely related to that of the Arachnid family which is known for its spinning capabilities.