From 2D to 3D: Using CLSM to Explore Theodore B. Mitchell's Collection of Bees

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Introduction

- Bees are essential pollinators in our food systems (1, 2).
- To better understand pollination services, we must be able to accurately identify bees on the species level (3).
- The armature surrounding the genitalia, known as terminalia, is distinctly different between bee species, making it an important reference for identification (4).
- Currently, the only reliable resources for most native North American bee identification are line drawings from Mitchell's "Bees of the Eastern United States" (5, 6).
- Imaging technologies have vastly improved since Mitchell's research.
- Today, morphological concepts can be better understood using 3D imaging technologies, including Confocal Laser Scanning Microscopy (CLSM, 7).
- **To explore details hidden in Mitchell's illustrations, we use CLSM to generate 3D data of the male terminalia from Mitchell's original collection.**

Figure 1: Mitchell's collection of Colletidae, Andrenidae, and Megachilidae specimens.

Figure 2: Lateral view of a *Colletes simulans* specimen (NCSU_0010038) from Mitchell's collection.

Methods

- In the 1960's, the male terminalia was dissected out of Colletidae, Andrenidae, and Megachilidae specimens and embedded in Canada balsam by Theodore B. Mitchell.
- Mitchell used these specimens as references for his illustrations.
- The Don Chandler Entomological Collection borrowed Mitchell's specimens from the North Carolina State University Insect Museum.
- The Nikon A1R-HD CLSM was used in the University of New Hampshire's Instrumentation Center and utilized the autofluorescence of the insect skeletomuscular system.
- Higher excitation and emission wavelengths (639 nm and 700 nm, respectively) were used to avoid signals from the autofluorescence of Canada balsam.
- Volume-rendered micrographs and media files were created using FIJI (8).

Figure 3: Mark Townley operating the Nikon A1R-HD CLSM at the University of New Hampshire's Instrumentation Center.

Mitchell's Illustrations

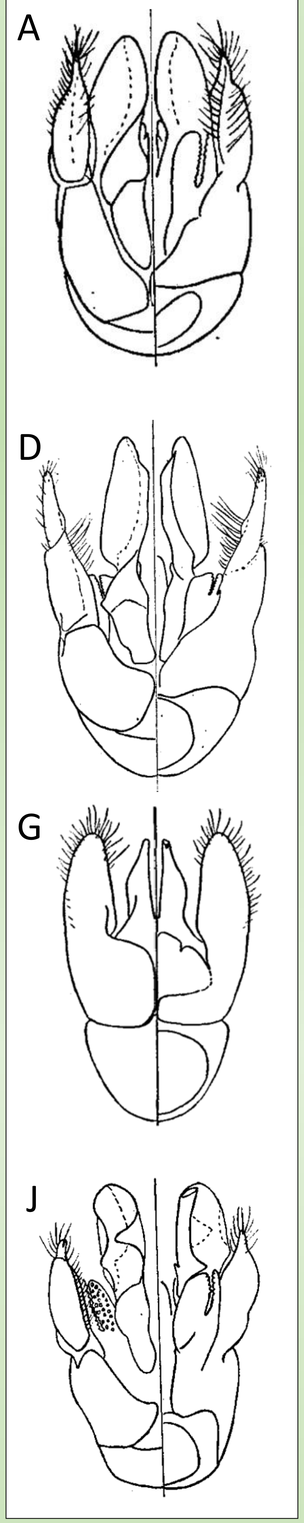

Figure 4: Mitchell's illustrations of *Colletes americanus* (A), *Colletes simulans* (D), *Hylaeus verticalis* (G), and *Colletes brevicornis* (J) terminalia. The left half is the dorsal view, and the right half is the ventral view.

CLSM Micrographs

Figure 5: CLSM micrographs of the terminalia of a *Colletes americanus* specimen (NCSU_0026013) (B, C), *Colletes simulans* specimen (NCSU_0010038) (E, F), *Hylaeus verticalis* specimen (NCSU_0013014) (H, I), and *Colletes brevicornis* specimen (NCSU_0025353) (K, L). Dorsal views are on the left and ventral views are on the right.

Comparing 2D to 3D

The **scientific illustrations** can highlight and emphasize the differences the descriptor finds the most notable or important for diagnosis. The illustrations provide a general model lacking intraspecific variation.

The **CLSM micrographs** are more proportionally accurate and look closer to what a scientist would be able to observe through a microscope. The micrographs also provide resolution and reveal spatial relationships that cannot be observed with a conventional light microscope. Additionally, a gallery of micrographs can represent intraspecific variation.

CLSM micrographs increase machine readability of phenotypic concepts (9) and allow scientists to discover structures that are not illustrated in line drawings. Scientific illustrations highlight phenotypic differences between species and can be more human-friendly.

What about Female Bees?

- Due to **antagonistic co-evolution**, interspecific differences in the structure of male bee terminalia are likely related to interspecific differences in the structure of female bee reproductive tracts (10).
- Correspondence between male and female bee terminalia has not been explored.
- 3D data from this study and newly collected Synchrotron based micro-CT data of both male and female bee terminalia (in collaboration with the ANKA Synchrotron in Stuttgart, Germany) is being used to reveal correspondence.

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