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Occurrence of a Gynandromorphic Bombus bimaculatus (Hymenoptera: Apidae) in Southeastern Ohio

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Occurrence of a Gynandromorphic *Bombus bimaculatus* (Hymenoptera: Apidae) in Southeastern Ohio

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Abstract

Herein, we introduce the first reported case of gynandromorphy in the bumblebee *Bombus bimaculatus* (Cresson) (Hymenoptera: Apidae), a relatively common North American species found east of the Mississippi River. The specimen was collected in Marietta, Ohio as part of a bee diversity assessment project for Washington County. Gynanders exhibit discrete male and female characters in a single individual. We discuss the potential causes of gynandromorphy exhibited by this specimen, which has differing antennal segments (12 and 13), facial maculation, abdominal hair coloration, and the presence of a corbicula – secondary sex characters that are characteristic for the genus *Bombus*.

The presences of intersexes and gynandromorphs among the Insecta appear to be exceedingly rare based on those reported to date (Michez et al. 2009), yet this may be due in part to a lack of careful investigation of insect specimens to make such discoveries. An intersex is a genetically uniform individual that is ambiguously neither male nor female (Narita et al. 2010). A gynandromorph is an organism that has both genetically male and female tissues which can be distinguished from one another based on the characteristics of the tissues (Morgan, 1916). Gynandromorphs are found in three main forms: bilateral, mosaic, or transverse (Michez et al., 2009). Bilateral gynandromorphy is defined as the individual being divided bilaterally with one half male and the other female (Michez et al. 2009). Mosaic gynandromorphy is a patchwork of male or female areas (Michez et al. 2009). Transverse gynanders are less common and have the head of one sex and the body of another sex (Michez et al. 2009).

Given that bees are sexually dimorphic, it is easier to notice gynandromorphs compared to species that do not show as many secondary sex characteristics. Further, given that identifications of many bee species require careful inspection of multiple features under high-powered magnification, these characteristics may be more likely to be noted than on other arthropod species. Interestingly, a review of gynandromorphy in Apoidea found that there have only been 109 reports of gynandromorphy divided between the 6 families of bees (Apidae, Colletidae, Halictidae, Megachilidae, Andrenidae, and Melittidae) (Michez et al. 2009). A more recent review has increased this number to 113 bee species divided between 29 genera (Hinojosa-Diaz et al. 2012). It does not appear that certain species are more prone to gynandromorphy; however, with so few reports it is difficult to say how common and widespread this phenomenon is among bees. To date, gynandromorphy is best documented in the genus *Megachile*, largely due to work by T. B Mitchell in the 1920s to1940s (Michez et al. 2009).

During a study aimed at assessing bee diversity and pollen loads in Washington County, Ohio, a single gynandromorph of the species *Bombus bimaculatus*

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(Cresson) was collected. Herein, we document the characteristics of this individual and discuss potential causes and implications of gynandromorphy.

Methods and Materials

Study Sites. Three sites were selected as part of a bee diversity assessment project in Washington County, Ohio (Barbara A. Beiser Field Station (BFS), the Marietta College Campus (MC), and the Washington County Career Center (WCCC)). Within each site, three transects were established, with each being 150 meters in length (Fig. 1). These study sites contained wooded areas as well as developed areas containing built structures, turf grass, and weedy forbs and trees. The transects established at BFS and WCCC were located within old field habitat bordered by forest edge. The MC transects were located within mown turf grass alongside a stream containing weedy and invasive plants along its banks.

Bee Collection. Bees were collected from the sites using three main sampling methods: pan trapping, blue vane traps, and hand netting. Pan trapping was conducted every 1-2 weeks on non-rainy days from April to October 2013. Ninety 96 ml soufflé cups half full of soapy water (blue Dawn dish soap, The Proctor and Gamble Co., Cincinnati, OH) were placed along each transect, with alternating colors of white, fluorescent blue, and fluorescent yellow (Guerra paints, New York, NY). Blue vane traps were used from August to October to catch larger bees such as *Bombus* and *Melissodes* which pan traps were less likely to collect (Geroff et al. 2014). One blue vane trap was established per transect and half filled with soapy water. Both the blue vane traps and pan traps were set before sunrise and collected 24 hours later; trapping with vane traps coincided with pan trap sampling during August - October. Hand netted bee samples were also collected. These collections took place three times in 2013: in April, July, and August. For hand collection, the surveyor spent 5 minutes walking each transect and hand collected any bees found using a sweepnet. Bees were collected within 5 meters of the transect.

Collected bees were returned to the laboratory and stored in 70% ethanol in a refrigerator until they could be processed. Once sorted from bycatch, the bees were washed, blown dry, pinned and labeled with their collection date, site, and transect. Bees were identified under a stereo microscope following Michener et al. (1994) for genus determination and Discoverlife.org (Droege and Orr, 2013) for current species identification. The gynander individual was discovered during the identification process.

Results

Description of the Gynander - *Bombus bimaculatus*. The gynandromorphic *Bombus bimaculatus* was hand collected 3 July 2013 at the Barbara A. Beiser Field Station along transect 1. This transect was on an access road that was also old field habitat bordered by forest edge. The transect was parallel to the Little Muskingum River which was 20 meters from the transect and prone to flooding.

We found several differences in our gynander, summarized in Table 1. This individual's right antennae had 12 segments (♀) and its left 13 (♂) (Fig. 2). At the base of the right antennae, predominantly black hairs were found (♀) whereas the bee had yellow hairs at the base of the left antennae (♂) (Fig. 2). The mandible maculation was hairy and difficult to see (♂) on the right and glabrous with the integument clearly visible (♀) on the left (Fig. 3a and b). The hind leg had a distinct corbicula (♀) on the left, but a more rounded hind leg (♂) on the right (Fig. 4a and b). The femur hair color was black (♀) on the left and yellow (♂) on the right. The abdominal segment T4 was all black (♀) on the left and black with interspersed yellow hairs (♂) on the right. The hair on the abdominal sternites was black (♀) on the left and yellow (♂) on the right. The reproductive characteristics are unknown due to the fragile character of

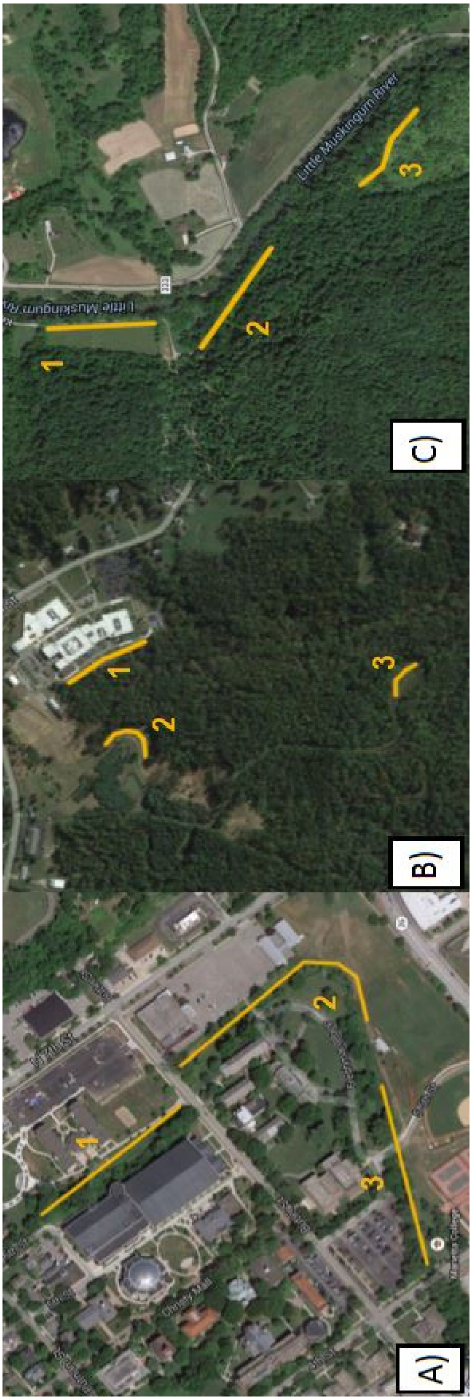

Figure 1. Transects at each of the sites. All transects are the same length (150 meters), but distances between them differ: A) Marietta College Campus. B) Washington County Career Center. C) Barbara A. Beiser Field Station (where the gynander was collected).

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Table 1. Characteristics present on the collected *B. bimaculatus* gynander compared with normal male and female characteristics for this species, based on the bees' left and right sides.

Character	**Female**	**Male**	**Gynander**
Antenna	12 segments	13 segments	Left ♂, Right ♀
Hair near antennae base	mainly black with a small patch of yellow	mainly yellow hairs	Left ♂, Right ♀
Mandible maculation	glabrous, integument clearly visible	hairy mandible, difficult to see integument	Left ♀, Right ♂
Legs	distinct corbicula on hind leg	rounded hind leg without corbicula	Left ♀, Right ♂
Femur - hair	black hair	yellow hair	Left ♀, Right ♂
Abdomen - T4 hair	all black	black with yellow mixed in	Left ♀, Right ♂
Abdomen - Sternites hair color	black	yellowish	Left ♀, Right ♂

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Figure 2. The gynander of *B. bimaculatus* exhibited differentiation in the number of antennal segments present, with 12 segments on the right (female) and 13 on the left (male). At the base of the antenna, black hairs predominate on the right (female) whereas on the left mainly yellow hairs are present (male). Left and right refer to the bee's left and right sides, not the photo.

the specimen. Since resecting the genitalia is not a common practice for normal bee identification, we did not perform this procedure upon initial pinning. The specimen has since become too brittle and frail to safely determine this.

Based on our observations, this bee does not fit in the category of bilateral. The sex does appear to be divided bilaterally, but it is not consistent across the entire bee. The male parts are on the right side of the abdomen and thorax, but for the head the male antennae and facial hair coloration is on the left side. However, the head is not perfectly divided either. The mandibles are hairy (♂) on the right side and glabrous (♀) on the left side. Thus, this bee is best categorized as mosaic because the pattern does not divide the entire organism in half.

Interestingly, another deformed bee was also collected as part of this project, but it was not a gynander. A *Ceratina strenua* specimen found to have deformed antennae with only 10 segments was also collected at the Beiser Field station. Otherwise, the *C. strenua* specimen appeared normal. Additionally, 6 intersexes of *Andrena* spp. were collected, but they had obvious Strepsiptera in their abdomens, a known cause of intersexuality.

Discussion

Gynandromorphs are known to be rare. Michez et al. (2009) compiled a list of the gynandromorphs in the literature, of which they only found 109 reported cases in the 6 families of bees worldwide. Our gynander was 1 of over 2,700 bees collected as part of a diversity assessment project in Marietta, Ohio. Although

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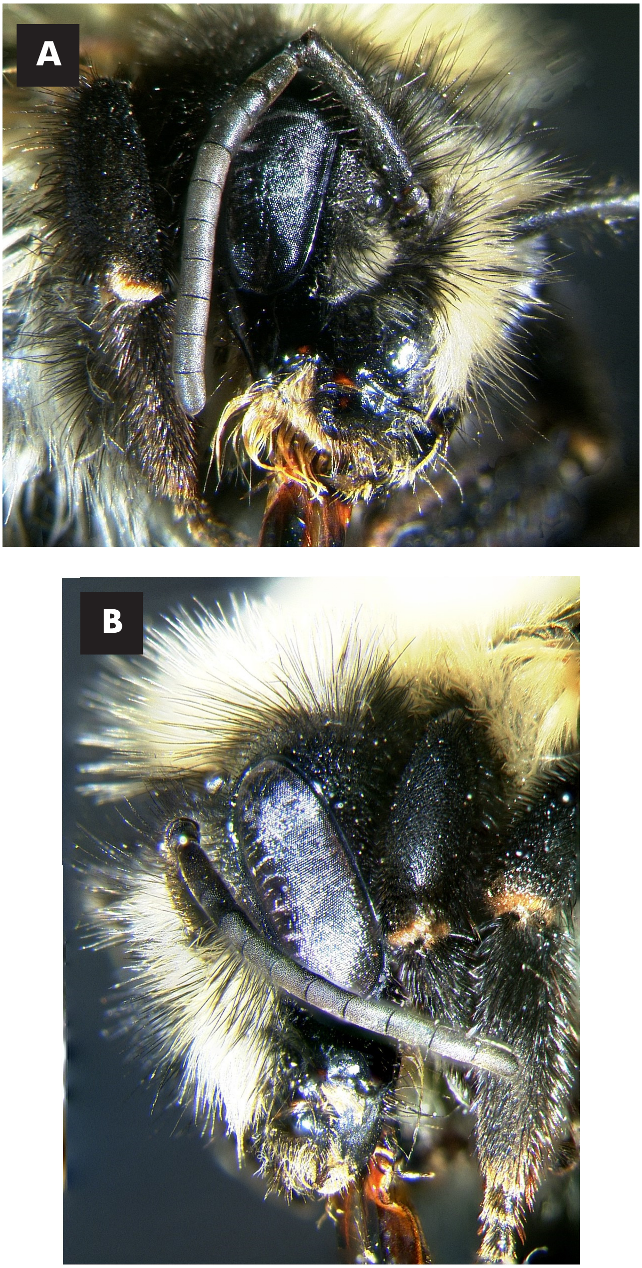

Figure 3. Sexual differentiation is more pronounced in the mandibles of *Bombus,* with the male having a smaller, hairy mandible (A) and the female having a larger, glabrous mandible (B).

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Figure 4. A distinct secondary sex characteristic in the *Bombus* genus is the presence of a corbicula in females. A) shows the female corbicula with a smooth hairless section in the center. B) reveals the opposite side of the gynander where a corbicula is distinctly lacking on the hindleg and is instead convex and covered with hairs.

multiple bees were collected with altered development, it is unlikely to be due to environmental contamination at the field station. The intersexes of *Andrena* spp. and reduction in secondary sex characteristics can easily be explained by the Strepsiptera parasitizing them (Salt, 1931). However, parasite infestation does not explain either the *B. bimaculatus* gynander or the *C. strenua* specimen with deformed antennae. Importantly, baselines in wild populations have not been established for developmental errors within bees, thus we do not have a basis for comparison. This was one of our motivations in reporting these individuals.

Several possible causes of gynandromorphy have been identified including chromosome elimination, polyspermy, postcleavage fertilization, and binucleate eggs (Narita et al., 2010). Errors in sexual determination play an important role in the production of gynandromorphs. For Hymenoptera, sex determination is based on haplodiploidy (Heimpel and de Boer 2008). This means that females are produced from fertilized eggs whereas males are produced from unfertilized eggs (Heimpel and de Boer 2008). Moreover, bees have a single locus complementary sex determiner (sl-CSD) where males are hemizygous and females are heterozygous for said locus (Crozier 1977, Heimpel and de Boer 2008). Thus, inbreeding in haplodiploid species with sl-CSD leads to the production of a male phenotype (Duchateau et al. 1994, Duchateau and Marien 1995).

Moreover, chromosome elimination can play a critical role in the expression of male or female sex. Chromosome elimination is the process of eliminating an entire set of chromosomes making a diploid individual into haploid (Michez et al., 2009). Failure to eliminate the chromosomes throughout the entire organism can lead to a patchwork of sexes in an individual (Michez et al. 2009). In the case of bees, the haploid tissue develops into the male areas and diploid into female tissues.

Polyspermy is an alternative explanation to creation of gynandromorphs. In this case, the egg is fertilized once to create a diploid female (Michez et al. 2009). However, with the additional sperm, there is a haploid nucleus that develops into the male parts of the organism (Michez et al. 2009). Dobata et al. (2012) found that mosaic gynandromorphy in an ant in the genus *Diacamma* was caused by either polyspermy or maternal genome elimination in the male tissues. Similar to polyspermy, postcleavage fertilization involves an issue with sperm. In this case, the sperm is unable to penetrate the egg until after the initial cleavage. This leads the fertilized section to become diploid with normal female characteristics whereas the unfertilized section becomes male tissue (Michez et al. 2009). Drescher and Rothenbuhler (1963) concluded that fertilization after the first cleavage was the cause of their gyndandromorphs when they chilled the eggs. Binucleate eggs could also explain gynandromorphy. If only one of the nuclei is fertilized, then the remaining nuclei will remain haploid and develop into male tissues.

It is unknown what caused this *B. bimaculatus* to be a gynandromorph. There are several possible explanations, but even with genetic testing, elucidating the specific error that lead to the creation of this gynandromorph would be challenging. Regardless of what caused it, this developmental anomaly is the first reported case of gynandromorphy in the species. Gynanders provide a unique aspect of field biology to understand the normal rate of developmental errors in the field instead of induced errors in the laboratory.

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