A scientific note on an anomalous diploid individual of *Euglossa melanotricha* (Apidae, Euglossini) with both female and male phenotypes

Karen M. SUZUKI¹, Douglas C. GIANGARELLI¹, Dhiego G. FERREIRA^{1,2}, Wilson FRANTINE-SILVA^{1,2}, Solange C. AUGUSTO², Sílvia H. SOFIA¹

¹Laboratório de Genética e Ecologia Animal, Departamento de Biologia Geral, CCB, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, km 380, 86057-970, Londrina, PR, Brazil

²Laboratório de Ecologia e Comportamento de Abelhas, Instituto de Biologia, Universidade Federal de Uberlândia, Campus Umuarama, Bloco 2D, 38400-902, Uberlândia, MG, Brazil

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In the current literature, there are over 100 reports of anomalous bees, showing both female and male phenotypes in the same individual, usually named gynander or gynadromorph (Wcislo et al. 2004; Michez et al. 2009). Curiously, almost 80% of the records of gynandromorphs are known from bee species of the Holarctic region belonging to the genera *Megachile* and *Andrena* (Michez et al. 2009; Hinojosa-Díaz et al. 2012). The scarcity of reports of gynandromorphism in bees from other world regions raises the question if the prevalence of gynandromorphs in bee species of some genera and from the Holarctic region is due to sampling bias or a true predisposition within those lineages toward gynandromorphism (Lucia and Gonzalez 2013). In face of that, records on gynander bees from other regions from the world are necessary to fill this gap.

The first records on gynandromorphism among the orchid bees (Apidae, Euglossini) in the Neotropics were

recently published. These reports involved four different species: *Euglossa iopoecila* Dressler (Giangarelli and Sofia 2011), *Euglossa tridentata* Moure (Hinojosa-Díaz et al. 2012), *Eulaema atleticana* Nemésio (Silveira et al. 2012), and *Euglossa pleosticta* Dressler (Camargo and Gonçalves 2014). In all these four reports, the male and female tissues were distributed patchily through the bee body, and the gynanders were included in the category of mixed or mosaic, which together with anterior-posterior, bilateral and transverse (dorsal-ventral) comprise the four categories of gynadromorph reviewed by Wcislo et al. (2004).

The origin of the gynandromorphs has been attributed to genetic problems, and although different hypotheses have been raised to explain genetically the origin of the gynandromorphism in bees, the mechanisms that generate these abnormal individuals have not been elucidated (see Michez et al. 2009).

This note brings, for the first time, findings obtained through both morphological and genetic analyses of a gynander orchid bee (*Euglossa melanotricha*). For the genetic analysis, microsatellite markers were used to genotype the gynander bee.

The gynander of *E. melanotricha* analyzed was collected after its emergence in a nest collected in an area of Cerrado (Brazilian savanna), located in the municipality of Uberlândia (18° 55' S; 48° 17' W), southeastern Brazil.

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Corresponding author: S. Sofia, shsofia@uel.br H. Sofia, shsofia@uel.br

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The morphological measures and description of the anomalous bee were performed according to Giangarelli and Sofia (2011). For the purpose of morphological comparison, we also analyzed a normal male and a normal female from the same nest. The morphological terminology and some of the standard morphological measures herein used follow that of Michener (2007) and Nemésio (2009).

A photo-stereomicroscope Discovery.V20 (Zeiss) was used for morphological and photographic records. In addition, two normal females, collected from the same nest as the gynander, and two normal males, from a different locality, were also genotyped (control).

DNA was extracted from the right and left hind legs of the bees, according to Freiria et al. (2012). In the anomalous bee, the right and left hind legs showed, respectively, the female (F) and male (M) phenotypes. Seven heterologous primers, described for *Euglossa cordata* (Souza et al. 2007) and *E. annectans* (Paxton et al. 2009), were used in PCR reactions. For the genotyping on an automated sequencer (ABI sequencer model 3500xl), the forward primers were prepared according to Schuelke (2000). PCR conditions, carried out in a 5- μ L volume and primer annealing temperatures are shown in Table I-S. GeneScan 600-LIZ (Applied Biosystems) was used as the standard marker. The sample genotypes were analyzed using the GeneMarker 1.85 software (SoftGenetics) and checked manually.

1. MORPHOLOGICAL DESCRIPTION OF THE GYNANDROMORPH (FIGURE 1A–K)

The measurements of the anomalous bee were (in mm): total body length, 11.2; intertegular distance, 3.52; head width, 4.82; right mandible, 1.64 (bidentate), left mandible, 1.24 (tridentate); antennae, 3.58 (right), 3.58 (left); and 12 segments with 10 flagellomeres. The measures of mandibles correspond to the largest axis, ranging from the base to the apex.

Most of the body showed female characteristics: paraocular unstained head and antennae with 12 segments, an ellipsoid scutellar tuft and a sting, which was also revealed by a partial dissection of the abdomen (Figure 1f). The metasoma with six exposed terga and sterna was apically narrowed. The right legs exhibited a female phenotype and the left legs, a male phenotype: (i) metatibia with a slit (left side) (male feature), (ii) metatibia with a corbicula (right) (female feature), (iii) mesotibia (left) with a velvety area with basal and distal

cushions (typical male characteristics), and (iv) mesotibia (right side) without velvety area and cushions.

Main morphological description of the normal male (M) and female (F), in mm: total body length, 11.69 (M), 11.81 (F); intertegular distance, 3.74 (M), 3.78 (F); head, left antennae (M)=13 segments, length (3.79); right antennae (F)=12 segments, length (3.87); mandible bidentate (M), 1.23 (left) and 1.24 (right); and mandible tridentate (F), 1.66 (left) and 1.64 (right). Legs (M and F) and other characteristics are in accordance to Nemésio (2009).

2. GENETIC ANALYSIS OF THE GYNANDROMORPH

The genotyping of both hind legs (left/M and right/F) of the anomalous bee showed exactly the same genotypes for male and female parts of the bee (Table II-S). From the seven loci amplified, five were heterozygous, indicating that the organism analyzed is compatible with a diploid organism and not with a hemizygous or haploid one.

The results revealed that the gynandromorph shared several alleles with both normal females analyzed as well as with the two control males (Table II-S). Both females were heterozygous for six loci. Males showed only one allele per locus, indicating they were haploid organisms. Sizes of the alleles identified ranged from 134 to 198 bp (Table II-S).

As in other reports on gynanders of orchid bees, the specimen of *E. melanotricha* analyzed herein was included in the category of mixed (or mosaic), apparently the most common category of gynandromorphism among bees (Wcislo et al. 2004). However, it is noticeable that the anomalous *E. melanotricha* showed a trend to partial bilateralism, since male phenotype was detected only in the left side of the body, i.e., the left bidentate mandible and legs. This same trend has already been noticed for gynanders of *E. iopoecila*, *E. tridentata*, and *E. pleosticta* (see Camargo and Gonçalves 2013).

The clear predominance of female tissues throughout the body of the gynander of *E. melanotricha* is another characteristic shared with the four gynanders of euglossines known to date. Besides, a metasoma exhibiting predominantly female phenotype and carrying a sting, as observed herein, has already been reported for *E. atleticana* (Silveira et al. 2012) and *E. pleosticta* (Camargo and Gonçalves 2013).

The heterozygosity detected for five of seven microsatellite loci and the same genotype for both tissues from the left (male trait) and right (female trait) legs of

Figure 1. Individual of *Euglossa melanotricha* with male and female phenotypic characteristics. **a** Side view (right), **b** side view (left), **c** front view of the head, **d** scutellum with a scutellar tuft (as observed in the female of this species), **e** view of the abdomen showing the sting, **f** ventral view of the sting, **g** dorsal view, **h** metatibia (left) with the presence of slit, **i** metatibia (right) with corbicula, **j** mesotibia (left) with velvety area and basal and distal cushions, **k** mesotibia (right side).

E. melanotricha indicate that the bee analyzed is a diploid individual. Thus, our results indicate that the gynander of *E. melanotricha* shows morphological and genetic (i.e., diploidy) characteristics predominantly of the female sex. On the other hand, when considering the genetic uniformity of phenotypically different tissues (male and female) of this individual, the gynandromorph of *E. melanotricha* seems to fit better in the category of intersex bee, in accordance with the definition presented by Narita et al. (2010).

Since the early twentieth century, different genetic hypotheses have been proposed to explain the causes of gynandromorphy in Hymenoptera (reviewed by Michez et al. 2009). Furthermore, with the advances in studies on sex determination in this group of haplodiploid

insects, new attempts have been made to explain the origin of gynandromorphs in the light of the recent findings, especially those involving the *csd* locus (see Michez et al. 2009).

Among euglossines, as in other groups of bees, the single locus complementary sex determination (sl-CSD), involving a multiallelic locus (*csd*), has been widely accepted as the system acting in sex determination (Zayed 2009). In normal conditions, heterozygotes at the *csd* locus develop into diploid females from fertilized eggs, while hemizygotes develop into haploid males from unfertilized eggs. Also, individuals homozygous at this locus develop into diploid males (Zayed 2009). It has been proposed that in gynandromorphs showing female and male diploid characters, female tissues have to be heterozygous in the *csd* locus, while

male tissues could be *csd* homozygous as consequence of mutation and/or inhibition in the *csd* allele (Michez et al. 2009). Besides, it has been also demonstrated that *csd* repression by RNA interference can produce male characters in genetic females (Beye et al. 2003). Thus, a plausible explanation for the diploid genotype detected in the male and female tissues of the euglossine bee herein analyzed would be a problem in the mechanisms of *csd* gene regulation during the development of this bee. However, at this moment, our results are not sufficient to prove or refute this hypothesis since our study on the anomalous *E. melanotricha* did not include any analyses about products of *csd*.

In light of the above scenario, we suggest that future studies on gynander and intersex bees should give more emphasis to the understanding of the mechanisms involved in the *csd* gene regulation in an attempt to better elucidate how these anomalous organisms are generated.

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Note scientifique sur un individu anormal diploïde d'*Euglossa melanotricha* (Apidae, Euglossini) présentant des phénotypes à la fois mâle et femelle

Eine wissenschaftliche Notiz über ein anormales diploides Individuum von *Euglossa melanotricha* (Apidae: Euglossini) mit sowohl männlichem als auch weiblichem Phänotyp

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