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Diploid Male Frequencies in Colombian Populations of Euglossine Bees

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ABSTRACT

Some studies have recently reported high diploid male frequencies in euglossine bees indicating possible population declines. We estimated the genetic variability and frequency of males that are diploid in five Colombian species of euglossine bees using allozyme markers. Expected heterozygosities ranged from 0.10 to 0.23. Diploid males were found in three species with frequencies ranging from 8 to 32 percent. Our results suggest that some species are more susceptible to environmental changes and anthropogenic pressure.

Abstract in Spanish is available at http://www.blackwell-synergy.com/loi/btp.

Key words: allozyme markers; Colombia; Euglossini tribe; genetic variability; heterozygosity.

SEX DETERMINATION IN MOST SPECIES OF HYMENOPTERANS IS CONTROLLED BY A MULTI-ALLELE SINGLE LOCUS (Beye *et al*. 2003). In this system, unfertilized eggs develop into haploid males, whereas fertilized eggs produce (1) females when heterozygous or (2) males when homozygous at the sex locus (Cook & Crozier 1995). Diploid males have a fitness value close to zero because they are either inviable or effectively sterile and thus constitute a severe genetic load to natural populations (Zayed & Packer 2005). However, large populations at the mutation–drift equilibrium are expected to maintain a large number of sex alleles, which minimizes the production of diploid males (Yokoyama & Nei 1979).

Euglossine bees (Apidae: Euglossini) are key species in Neotropical ecosystems due to the pollination services they provide to a large number of plant species. Richness and abundance of euglossine populations have been extensively studied using chemical baits that mimic the fragrances usually collected by males from flowers and other sources (Roubik & Ackerman 1987, Roubik 2001, Tonhasca *et al*. 2002). In the last decade, interest in this group has grown, due to the high frequencies of diploid males found in some populations. A study of allozyme variability among nine Panamanian species found diploid male frequencies ranging from 12 to 100 percent and it was suggested that all euglossine bees potentially have high proportions of diploid males (Roubik *et al*. 1996). However, a study of 14 euglossine bee species of Brazil found only one diploid male among the 568 individuals analyzed (Takahashi *et al*. 2001).

The apparent contradiction between the extremely high frequencies of diploid males in Panamanian populations and their absence in Brazil remains unexplained. To shed new light on these puzzling results, we estimated the genetic variability and diploid male frequency in populations of five species of Colombian euglossine bees from two different habitats. Diploid males were found in three out of the five species analyzed. We believe these findings are the result of anthropogenic pressure as well as ecological and demographic features of the species. Furthermore, the results of this study also highlight the need for conservation efforts for some vulnerable native bee species.

Sampling of male euglossine bees was performed from 0900 h to 1300 h using nine chemical baits (cineole, methyl salicylate, dimethoxy benzene, vanillin, methyl cinnamate, eugenol, benzyl acetate, phenyl ethyl acetate and skatol) on papers fastened to tree trunks located 50 to 200 m in the forest. Bees were collected with entomological nets, immediately placed in plastic vials on ice during the fieldwork and later kept in a freezer at −80°C until analysis.

Genetic variation was assayed with allozymes in horizontal starch gel electrophoresis. Proteins were extracted by macerating the head and thorax of captured specimens in a 0.2 percent 2-mercaptoethanol solution. We used 11 enzyme systems: adenylate kinase, AK (TC 8.0); aldehyde oxidase, ALO (TVB 8.5), aldolase, ALD (TEMM 7.4); malic enzyme, ME (TC 8.0); esterase, EST (TVB 8.5); phosphoglucomutase, PGM (HTC 6.0-6.6); α-glycerophosphate dehydrogenase, GPDH (TC 8.0); hexokinase, HK (TEMM 7.4); malate dehydrogenase, MDH (TC 8.0); peptidase leu-tyr, PEP (RW 8.5) and pyruvate kinase, PK (RW 8.5). All buffers and reaction mixtures were prepared according to procedures described by Harris and Hopkinson (1976).

Samples of the species *Eulaema cingulata, Euglossa piliventris, Eg. prasina* and *Eg. singularis* were collected in June and July 2003 in a humid tropical forest at the Cerca Viva natural reserve in the Colombian Amazon region (04°07′14″ S, 69°57′07″ W). This reserve is a conservation area with little human intervention, located 11 km north of the city of Leticia. *Eufriesea magrettii* was collected in a grassland foothill ecosystem at Vereda del Carmen (4°09′00″ N,

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73°39′30″ W) during September 2003. This site is located close to the city of Villavicencio, in an area under threat from urbanization and road construction.

Because of the presence of both haploid and diploid males among the individuals we sampled, allelic frequencies for each locus were calculated as the number of occurrences of an allele divided by the total number of alleles at that locus. Intra-locus heterozygosity (H_i) was calculated as $H_i = 1 - \Sigma p^2$, where p is the frequency of each of the alleles present at the locus. The expected average heterozygosity ($\overline{H}_{\text{exp}}$) was estimated over all loci as $\Sigma H_i/n$ assuming Hardy–Weinberg equilibrium and equal allele frequencies between sexes, where n is the total number of loci analyzed.

Frequency of diploid males (ϕ) was estimated for each locus using the Owen and Packer (1994) maximum likelihood model for two alleles using male data only as $\phi = B_2/2pqT_2$, where B_2 is the number of diploid males, $2pq$ is the frequency of heterozygotes and T_2 is the number of males sampled. To test the power of markers for estimating diploid males, the probability of one sampled male, if diploid, being heterozygous for at least one polymorphic locus (P_{het}) was calculated (Takahashi *et al*. 2001).

A total of 12 loci were sampled of the 11 enzyme systems analyzed. Despite showing interspecific variation, three loci were monomorphic (95% criterion) for all five species: ALD, AK, and PK. Expected heterozygosities ($\overline{H}_{\text{exp}}$) ranged from 0.10 to 0.23, with *Ef. magrettii* being the least variable and *Eg. prasina* the most variable species (Table 1). However, the difference in $\overline{H}_{\text{exp}}$ did not differ between species ($t = 1.28$; df = 7; $P > 0.05$, n.s.) (Nei & Kumar 2000).

Nine diploid males were detected from the results obtained with the EST and PGM loci: five in *El. cingulata*, three in *Ef. magrettii* and one in *Eg. piliventris*, corresponding to mean diploid male frequencies of 16, 32, and 8 percent, respectively (Table 1). One individual of *El. cingulata* was heterozygous for both loci. The probabilities of detecting a male, if diploid, with the markers used in this analysis were high for all species (0.79–0.98). Considering the sample size and intra-locus heterozygositis, the expected maximum frequency of diploid males for *Eg. prasina* and *Eg. singularis* were 12 and 9 percent, respectively (Owen & Packer 1994).

TABLE 1. *Population parameters of the five euglossine bee species analyzed.* N, *number of sampled males;* H_i, *intra-locus heterozygosity for variable loci;* H_{exp} *mean expected heterozygosity; ø, estimated frequencies of males that are diploid (± SD);* P_{het}, *probability of one sampled male, if diploid, being heterozygous for at least one polymorphic locus.*

Species	N	H_i	$\overline{H}_{\text{exp}}$	$\phi \pm$ SD	P_{het}
El. cingulata	50	0.08–0.47	0.13	0.16 ± 0.089[a]	0.90
Eg. piliventris	33	0.13–0.44	0.14	0.08 ± 0.079	0.90
Eg. prasina	17	0.37–0.50	0.23	–	0.95
Eg. singularis	25	0.27–0.63	0.20	–	0.98
Ef. magrettii	17	0.01–0.49	0.10	0.32 ± 0.169	0.79

[a]Average frequency of diploid males calculated from EST (0.13) and PGM (0.19).

The genetic variability we found in Colombian populations was lower than that previously reported for Panamanian populations of *Eg. imperialis* ($H_{\text{exp}} = 0.26$; Zayed *et al*. 2004), but higher than for Brazilian populations ($\overline{H}_{\text{exp}} = 0.01-0.07$; Takahashi *et al*. 2001). This difference is probably due to differences in the number and type of allozymes used in each study. The two most variable loci in our study were EST and PGM, which were two of the three used by Zayed *et al*. (2004) in Panama. In contrast, Takahashi *et al*. (2001) analyzed a higher number of loci (20 to 28 loci), seven of which were monomorphic for all species, and three were polymorphic for only one of the 16 species analyzed. The small and nonrandom sampling of allozyme loci can overestimate genetic variability estimates in population genetics studies.

The highest diploid male frequency (32%) and the lowest genetic variability we found corresponded to *Ef. magrettii*, a rare (Ramírez *et al*. 2002) and seasonal species (López-Uribe, pers. obs.). Although low genetic variability could be a feature of this species, the high frequency of diploid males suggests genetic erosion. We believe that loss of genetic variability could be due to isolation and degradation of euglossine habitats through anthropogenic pressures.

The species from the Amazon region showed lower levels of diploid males (0–16%) as expected from a less disturbed and continuous habitat. *Eulaema cingulata* and *Eg. piliventris* showed the lowest values of expected heterozygosity and showed diploid males, reinforcing the relationship between genetic variability and diploid male production. *Eulaema cingulata* was also analyzed by Roubik *et al*. (1996) in Panama where this species was found to be monomorphic and therefore the frequency of males that are diploid was not estimated. Takahashi *et al*. (2001) found no diploid *El. cingulata* males in a sample of 39 individuals from six different sites. As the area sampled in our study is preserved, our genetic results and previous ecological reports (Tonhasca *et. al.* 2002) suggest that *El. cingulata* disperses better through open areas rather than through closed forest. In that case, the high abundance of this species during our surveys could be explained by one or a combination of the following: (1) sampling during an abundant period of the species; (2) bias regarding the chemical baits used, which this species may have especially preferred; or (3) an overestimation in male census data given that production of diploid males increases the proportion of males sampled in a population. The latter also highlights the masking effect of censuses of euglossine male bees based on chemical baits.

Mark-recapture (Janzen 1971) and phylogeographic data (Dick *et al*. 2004) indicate that euglossine bees have long-distance dispersal ranges. This could be important for population maintenance of these bees as a way of avoiding inbreeding. However, fragmentation and habitat degradation may have severe effects for some species since euglossine bees appear to differ in their flying capacities over open areas (Milet-Pinheiro & Schlindwein 2005).

Although diploid male production is a population parameter, some species could be more vulnerable to this phenomenon. For example, Otero and Sandino's (2003) surveys across a human intervention gradient found that *Eg. imperialis* was more frequent in secondary forest than in farmlands. However, genetic and ecological data suggest that some Panamanian populations of this species are

declining (Roubik 2001, Zayed *et al*. 2004). Therefore, *Eg. imperialis* is probably susceptible to isolation by habitat fragmentation, which causes loss of genetic variability. In contrast, *El. nigrita* and *Eg. cordata*, the best sampled species by Takahashi *et al*. (2001) in Brazil, have high tolerance to disturbed areas (Tonhasca *et al*. 2002, Milet-Pinheiro & Schlindwein 2005) and may therefore be less likely to suffer negative effects of population isolation. Similarly, low genetic variability, marked seasonality, and restricted distribution of *Ef. magrettii* make this species highly susceptible to environmental changes and habitat fragmentation resulting from anthropogenic activities. Nonetheless, wider sampling is needed for conclusive results, the data presented here support the hypothesis of diploid male frequencies as an indicator of population decline in Hymenoptera (Zayed *et al*. 2004).

Bee diversity is nearly unknown for highly diverse countries such as Colombia, where there is a lack of taxonomic bee research (Nates-Parra & González 2000). Despite this, Colombia harbors the highest number of euglossine bee species and many remain to be described (Ramírez *et al*. 2002). Taxonomic identification and biogeographical information of bees are necessary for conservation efforts to focus on preserving vulnerable native species in biodiversity hotspot areas. Hence, studies on biological and genetic bee diversity should be a conservation priority, since habitat destruction occurs at high rates.

Our results show that diploid males do exist in Colombian euglossine populations, though at lower frequencies than in Panamanian populations. As mentioned above, we believe that high diploid male production is not a general trait of euglossine bee populations but a phenomenon result due to a combination of: (1) loss of habitat quality and connectivity among suitable fragments; and (2) particular biological, demographical, and genetic features of the species. Less variable and geographically restricted species are more likely to produce diploid males and are therefore vulnerable to population decline through isolation and habitat degradation. We hope these results will be considered in achieving realistic solutions for the pollination decline problem and bee conservation programs.

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