

## SEARCHING FOR GENETIC PATTERN IN ORCHID BEES: A REPLY TO TAKAHASHI ET AL.

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The Euglossini are Neotropical forest bees who manage to find and pollinate a number of rare plants (as well as finding and mating with rare conspecifics; Williams 1982). Our study in Panama during 1991 (Roubik et al. 1996) was the first to investigate population genetic traits in this group. Remarkably, we found that 55 of the 199 males assayed in four of nine principal species were diploids. We scrutinized the gels for possible errors of interpretation but found none. Although male Hymenoptera are haploid, some bizarre diploid males are viable and possibly fertile when homozygous at the sex-determining locus. A small percentage of them normally occur in honeybee colonies, where they are eliminated by the workers before reaching maturity. Less advanced social bees like halictids and bumblebees have been found to have diploid males present in populations experiencing inbreeding or a loss of allelic variation (Packer and Owen 2001; Duchateau and Mariën 1995).

Takahashi et al. (2001) report intriguing results from Brazil that seem to contradict our findings. In particular, they find that Euglossini there have few diploid males in a total sample comparable to ours from Panama. Spotty studies on such a varied group (200+ species) are bound to provide an incomplete picture. Nevertheless, this picture may have been blurred by the reanalysis of diploid male euglossines by Takahashi et al. First, both studies, in part, reach similar conclusions: that Euglossini are no different than other bees without advanced sociality or large colonies. Euglossine bees, whether in South America or Panama, show expected heterozygosity and polymorphism twice that of the related advanced social species in the same subfamily. This fact alone may be important in constraining the development of social behavior and the evolution of permanent colonies in that individuals may be too genetically diverse to work as an integrated whole (Roubik et al. 1996). Secondly, and perhaps more important, this comparison involves mostly different species. A more ideal comparison involving the same species in Brazil and Panama would be possible as about one-fourth of the 66 species in Panama also live in Brazil. Three species were shared between the two studies. In one of the smallest euglossines, *Euglossa sapphirina*, diploid males were found in Panama but no isoenzyme variability was detected in Brazil (perhaps due to the small sample size,  $n = 3$ ). In *Eulaema nigrita*, one diploid male was found in Santa Rosa National Park, Costa Rica, but none was found in Panama ( $n = 6$ ) or Brazil ( $n = 183$ ). Finally, in *Eulaema cingulata* no diploids were found in either area ( $n = 58, 39$ ), but this species was monomorphic in Panama in the several enzyme systems we ran. These results suggest that direct karyotypic analysis is preferable to isoenzyme analysis where variation is slight (Roubik et al. 1996).

Phylogeography could conceivably explain wide differences in genetic structure and propensity to produce diploid males among populations from different areas. For example, Euglossini that have adjacent montane and dry habitats can migrate during wet or dry seasons (Janzen et al. 1982), possibly avoiding inbreeding or undergoing seasonal shifts in genetic structure. In contrast, highly endemic species or those subject to habitat fragmentation or dispersal barriers might tend to be inbred and thus more likely to produce diploid males. As male mating success depends on their ability to collect rare chemicals (Eltz et al. 1999), there might also be strong sexual selection favoring only a few highly successful male lineages. (Multiple mating has yet to be demonstrated in euglossines.) Such reductions in effective population size might create genetic bottlenecks that could drive local populations toward extinction. Because I was concerned that chemical baiting of males might give a few males special advantages, contributing to inbreeding, I suspended monthly baiting studies in Panamanian forests after 1994.

Hymenoptera are genetically relatively monomorphic compared to other insects, and the males are often scarce. The reason for our work rested in part on the ease with which males could be collected in the field. Despite large samples in Panama, the lack of polymorphic loci in two genera blocked our ability to detect diploid males. The other two genera produced 3–54% male diploids (as detected in three polymorphic enzyme systems). We attempted to obtain large population samples in Panama ( $n = 60$ ) but we were limited by particular starch gel systems for certain loci.

In the Brazilian study, only three species were sampled in numbers comparable to our survey of nine species in the four genera. Sampling few males for allozyme analysis will often result in finding no diploid males, limiting the power to test gene frequency models. Takahashi et al. (2001) found only one diploid male among their larger samples of two polymorphic species in Brasil (28 loci scored). A male of *Euglossa mandibularis* was diploid, but it was the only such bee of over 500 individuals and 16 species assayed for electrophoretic variants sampled using the chemical attractants we all employ in field studies (Williams 1982). In contrast to our work in Panama, we suspect that there were not multiple male progeny of individual parents sampled in Brazil. Our work in Panama sometimes included a few dozen males taken at the same time in species that had male diploids, potentially violating the maximum-likelihood sampling assumption made in the calculations from Owen and Packer's (1994) formula for estimating diploid frequency among diploid males that include homozygotes. Our study may have erroneously concluded that male vagility would render such a sampling condition unlikely (Roubik et al. 1996). Nonethe-

less, we found unexpectedly high numbers of diploid males among Panamanian euglossines, and because of very low genetic polymorphism and the subsequent limitations of protein electrophoresis, frequencies of diploid males could not be determined in half of the genera sampled. There is no evidence of social behavior or colonies in cleptoparasitic *Exaerete* and communal *Eufriesea* in contrast to colonial, weakly eusocial *Euglossa* or *Eulaema*. This led us to suggest an evolutionarily resilient hypothesis to explain the correlation between genetic variation and this life-history trait: polymorphic females and abundant diploid males would diminish some advantages for cooperation (e.g., sacrificing personal reproduction), discouraging colony formation.

Euglossini may be particularly vulnerable to habitat fragmentation (Janzen 1981; Peruquetti et al. 1999). In the Brazilian species with large samples (Takahashi et al. 2001), the *Eulaema* and *Euglossa* are in fact "weedy" species and are able to survive in marginal or disturbed habitats, as known from Brazil and Panama (Roubik 1993; Peruquetti et al. 1999). In contrast, bees studied in Panama were largely deep forest species, associated with high population stability and predictable seasonality (Roubik 2001). Despite these outward signs of health, some local populations can now be postulated to show genetic degradation by producing so many functionally sterile males (Packer and Owen 2001). There may also be fundamental differences in the reproductive genetic engineering of species adapted to very different kinds of environments. Bee populations at risk from inbreeding might display behavioral adjustments to increase outbreeding, including multiple mating and the postulated but unproven lek displays of euglossine males. This insight is also beneficial to the controversy that currently exists over the number of matings that meliponine queens undertake (Peters et al. 1999).

What can we make of these scattered observations? When the results of our study were taking shape we speculated that the Hymenoptera were not as well known as we had assumed (see Butcher et al. 2000). Indeed, the possibility of reproductively viable diploid males emerged in a later, unpublished study by T. Eltz and D. Roubik with *Euglossa* reared in cages and given chemical compounds before the males had an opportunity to court females. Male *Euglossa* (a species not included in the report of Roubik et al. 1996) that later displayed at their perches and exhibited normal courtship behavior were, according to electrophoretic assay after the behavioral observations, also diploid in some instances. Again, we question the wisdom of applying one genetic sex determination model to all Euglossini. If some species have more than one sex-determination locus, then they will remain less likely to produce "useless" diploid males, compared to a population with a single sex-determination locus and, perhaps, a severely limited number of alleles. Even as we review

the biology of Euglossini, it is apparent that some findings are confused and poorly explained. Thus, conclusions from either Roubik et al. (1996) or Takahashi et al. (2001) should be cautiously considered. Certainly all Euglossini are not the same, including the individuals of one species. An interesting shift of emphasis in field work on euglossines might be to determine whether fragrances collected by males allow a diploid male to contribute in some way to viable matings.

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