

SHORT COMMUNICATION

Sex Ratio and Nest Observations of *Euglossa hyacinthina* (Hymenoptera: Apidae: Euglossini)

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Euglossa is the largest genus of orchid bees (Hymenoptera: Apidae: Euglossini), with 103 described species from northern Mexico to Paraguay and northern Argentina (Michener 2000; Roubik and Hanson 2004). For many species of these colorful, large-bodied insects females have not been identified. Males generally visit orchids to collect floral oils (see Eltz *et al.*, 1999; Eltz *et al.*, 2003), consequently, considerable information is available concerning male population biology, based on trapping data using artificial baits (reviewed in Roubik and Hanson 2004; Cameron 2004; Roubik 1989). Females do not exhibit such predictable behaviors at artificial baits; they visit a variety of flowers while collecting pollen, nectar, and resins, but they do not visit orchids regularly, and their biology is generally not well known.

Most *Euglossa* species are usually considered to be solitary (Ramirez-Arriaga *et al.*, 1996; Cameron 2004), but the presence of more than one female in the nests of some species indicates some form of sociality (Eberhard 1988; Soucy *et al.*, 2003, Augusto and Garófalo 2004). The general life cycle and natural history of *E. hyacinthina* Dressler is described by Eberhard (1988). Information on social behavior and brood sex ratio of this species is given by Soucy *et al.* (2003), based on nests collected during the late wet season and early dry season (late November to mid-February). The latter study showed that the numerical sex ratio of brood was not significantly different from one:one (female: male), and that numerical sex ratios could be used to infer investment sex ratios because there were no significant differences in body size (as measured by head width) between males and females. This note provides additional details on social biology and sex ratio based on nests of the same species at the same site and time of year. In contrast to Soucy and colleagues (2003), we show that numerical sex ratios are female biased, and that this measure may not accurately reflect investment ratios because females are significantly larger than males using inter-tegular distances, though are not different as measured by head width or wing length.

Materials and Methods

Study Site: We studied nests of *Euglossa hyacinthina* at the species' type locality in cloud forests near Fortuna, Panama (Chiriqui Province), at approximately 1400 m above sea level, where nests are abundant between the Jorge L. Arauz Center of Investigation (Smithsonian Tropical Research Institute) and the Edwin Fabrega Dam, along the roadsides between Los Planes and Chiriqui Grande.

Nest Collections: Nests of *E. hyacinthina* were identified during the day and collected at night, when all the occupants were sealed inside, between November 2001 and February 2002. Bees that emerged from the nest in the morning were identified as 'resident bees.' The nest interiors were observed with an otoscope and flashlight. Other individuals emerging later were identified as 'emerged bees.' The nests were transported to the Smithsonian Tropical Research Institute's Research Laboratory in Gamboa (Colon Province) and set in emergence chambers made of hardware cloth in an air-conditioned laboratory. The nests were monitored daily for emerging bees, which were collected from the chambers. We recorded the sex and emergence date of all bees from cells in each nest. From these observations, we calculated sex ratio data for the collected nests, using observations of 67 females and 22 male emerged bees. The overall sex

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Table 1. Emergence sex ratios for 30 nests of *E. hyacinthina*. Values given in bold are significantly different ($p < 0.05$) using a $\chi^2_{.05[1]}$ test against an expected sex ratio of 1:1.

	Ratio F:M	Proportion*	χ^2 Value
First Emergence	30:0	1	11.26, $p < .05$
Second Emergence	26:2	0.93	20.6, $p < .001$
Third Emergence	17:3	0.85	9.80, $p = 0.002$
Fourth Emergence	14:3	0.82	7.12, $p = 0.008$
Fifth Emergence	4:7	0.36	0.82, $p = 0.37$
Sixth Emergence	7:2	0.78	2.78, $p > 0.05$
Seventh Emergence	2:3	0.40	0.20, $p > 0.05$
Eighth Emergence	2:2	0.50	0, $p > 0.05$
Ninth Emergence	2:1	0.67	0.33, $p > 0.05$
All Emergences	104:23	0.82	8.96, $p < .05$

* Expressed as proportion females.

ratio was compared to a one:one ratio using binomial probabilities. All sex ratios are expressed as female proportions. We also quantified the body size differences between emerged males and females. Means are given with their standard deviations.

Data Analyses: We characterized body size using three variables: inter-tegular distance (ITD), forewing length (WL), and head width (HW). The sex ratio for each emergence position (first, second, etc) was calculated and compared to a one:one female to male ratio using binomial probabilities. All ratios are expressed as female proportions (Table 1)

Results

Nest descriptions

Forty-eight, aerial, resin, top-shaped nests (Eberhard 1988) were collected within 10 m of the road's edge (Fig. 1). Nearly all nests were found high on the main stem of the composite plant, *Bacchus* sp., and none were found within 5 m of one another. Nests were usually located away from overhanging vegetation in sun-lit areas, between 1 and 2.5 m from the ground, although some nests were found on vegetation on



Fig. 1. Resin nest of *E. hyacinthina* under construction on stem of *Bacchus* sp. (Asteraceae) along roadside in Fortuna, Chiriqui Province, Panama.

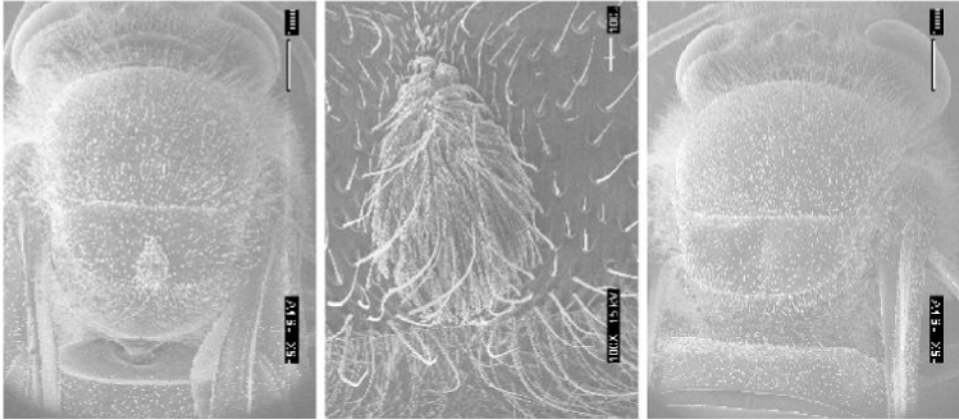


Fig. 2. Scanning electron micrographs of newly emerged *E. hyacinthina* A) female thorax B) close up, female thorax showing the patch of hairs on the scutellum C) male thorax.

steep slopes, as described by Soucy *et al.* (2003). Newly constructed nests, indicated by pliable resin walls, were almost orange in color and semi-translucent, with the overall appearance of a drying *Bacchus* leaf; older nests became hardened and darker brown, and on average were 58.9 ± 7.2 mm in length, and 42.2 ± 4.8 mm in width at the widest part. The nest entrances were usually circular openings 6.45 ± 0.55 mm in width constructed with a resin lip. Nests with active bees inside were usually closed with a pliable resin curtain overnight.

Nest contents

Of 30 productive nests, 7 had no adult bees present when they were collected. Fourteen nests contained a solitary female, while 9 nests contained more than 1 female. Of these nine, 4 nests had 2 females, 3 nests contained 3 females, and 2 nests contained 4 females. Of the nests collected, 18 did not contain or produce orchid bees; these nests typically contained colonies of ants, spiders, or mold.

Body size

Female bees have larger thoraxes than male bees. Body sizes for 68 females and 16 males were considered separately and compared to one another. Females tended to be larger than males, but these differences were significant only for ITD (Student's T, $P < 0.001$; Females: ITD = 4.90 ± 0.17 mm; WL = 9.38 ± 0.38 mm; and HW = 4.70 ± 0.18 mm. Males: ITD = 4.74 ± 0.13 mm; WL = 9.36 ± 0.35 mm; HW = 4.62 ± 0.13 mm). Female thoraxes have a teardrop shaped region of elongated, plumous hairs on the dorsal rim of the scutellum; males do not have this structure (Fig. 2).

Sex Ratio

Overall emergence sex ratio is highly skewed toward females, which comprised 82% of the population. The sex ratio was significantly different from 1:1 ($\chi^2_{.05[1]} = 51.66$, $P < 0.05$, $N = 127$). Nests that contained a single female when collected produced an average of 2.40 females, while nests that contained more than one female when collected produced an average of 1.66 females. The number of males produced by single female or multiple female nests did not differ.

Emergence sex ratios were significantly biased toward females for nests with more than one emergent bee. The first bees to emerge from the nests were females. There were 11 nests with at least five emergent bees; for each of these nests, the sex ratio differed significantly from one:one based on a Pearson Chi-Square ($\chi^2_{.05[4]} = 19.57$, $P > 0.002$)

Discussion

Based on this analysis, *E. hyacinthina* produced more females than males. In social Hymenoptera where singly mated females reproduce and non-mating females work, a female biased sex ratio is predicted (Trivers and Hare 1976) but solitary females, or those living in groups in which all females are

reproductively active, are predicted to have equal investments in females and males. Furthermore, the observation of significant differences in body size (inter-tegular distance) suggests that resource investment per female is greater than per male. It is likely that the inter-tegular distance is a more accurate measurement of body size differences than head width. Both of these findings differ from those reported by Soucy *et al.* (2003).

There are a few factors that might have affected our observations. Sex ratios in solitary bees and wasps are usually skewed depending on a variety of factors, including seasonal effects, brood mortality, weather conditions, and nest substrate availability (Michener 1974; Longair 1981; Strohm and Linsenmair 1997). It is not known if these factors affected *E. hyacinthina*. Another possibility is that there was some form of brood mortality due to transportation from the field to the laboratory; changes in temperature could have impacted the growth and development of young larvae and thus affected the sex ratio of the bees that emerged. We made every effort to standardize the environmental conditions during their transport to the laboratory. Finally, it is possible that our nest collections interrupted the nesting activities of the nest owners, which may have unwittingly skewed the emergence data. Only with further examination of this species will the possibility of these potential impacts be understood.

Mated female orchid bees can control the sex of their offspring; the sex ratio is therefore subject to evolutionary pressures. The factors that affect a female's decision to fertilize an egg are unknown (Michener 1974). Some observations of sex ratios are inconsistent and not related to sociality, but one could predict that a decreasing percentage of males should accompany increasing sociality (Michener 1974). Detailed studies are needed to understand social organization in this species; our observations suggest that further studies are warranted.

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Literature Cited

- Augusto, S. C., and C. A. Garófalo. 2004. Nesting biology and social structure of *Euglossa* (*Euglossa townsendi* Cockerell (Hymenoptera, Apidae, Euglossini). *Insectes Sociaux* 51:400–409.
- Cameron, S. A. 2004. Phylogeny and biology of neotropical orchid bees (Euglossini). *Annual Review of Entomology* 49:377–404.
- Eberhard, W. G. 1988. Group nesting in two species of *Euglossa* bees (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society* 61(4):406–411.
- Eltz, T., D. W. Roubik, and M. W. Whitten. 2003. Fragrances, male display and mating behavior of *Euglossa hemichlora*: a flight cage experiment. *Physiological Entomology* 28(4):251–260.
- Eltz, T., W. M. Whitten, D. W. Roubik, and K. E. Linsenmair. 1999. Fragrance collection, storage, and accumulation by individual male orchid bees. *Journal of Chemical Ecology* 25(1):157–176.
- Longair, R. W. 1981. Sex ratio variation in Xylophilous Aculate Hymenoptera. *Evolution* 35(3):597–600.
- Michener, C. D. 1974. *The Social Behavior of the Bees: A Comparative Study*. Harvard University/Belknap Press, Cambridge, Massachusetts. xi + 404 pp.
- Michener, C. D. 2000. *The Bees of the World*. Johns Hopkins University Press, Baltimore, Maryland. xiv + [1] + 913 pp.
- Ramirez-Arriaga, E., J. I. Cuadriello-Aguilar, and E. M. Hernandez. 1996. Nest structure and parasite of *Euglossa atroveneta* Dressler (Apidae: Bombinae: Euglossini) at Union Juárez, Chiapas, Mexico. *Journal of the Kansas Entomological Society* 69(2):144–152.
- Roubik, D. W. 1989. *Ecology and Natural History of Tropical Bees*. Cambridge University Press, New York, New York. x + 514 pp.
- Roubik, D. W., and P. E. Hanson. 2004. *Abejas de Orquídeas de la América Tropical*. Instituto Nacional de Biodiversidad, Santo Domingo de Heredia, Costa Rica. 370 pp.
- Soucy, S. L., T. Giray, and D. W. Roubik. 2003. Solitary and group-nesting in the orchid bee, *Euglossa hyacinthina* (Hymenoptera, Apidae). *Insectes Sociaux* 50(3):248–255.

- Strohm, E., and K. E. Linsen Mair. 1997. Low resource availability causes extremely male-biased investment ratios in the European beewolf, *Philanthus triangulum* F. (Hymenoptera: Sphecidae). *Proceedings of the Royal Society of London, Series B. Biological Sciences* 264(1380):423–429.
- Trivers, R. L., and H. Hare. 1976. Haplodiploidy and the evolution of the social insects. *Science* 191:249–263.