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POPULATION GENETICS, DIPLOID MALES, AND LIMITS TO SOCIAL EVOLUTION OF EUGLOSSINE BEES

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Population genetics using allozyme electrophoresis of four euglossine bee genera in Panama revealed high proportions of diploid males among social species but no genetic polymorphism in seasonal and parasitic species, thus, no possibility for discrimination of diploid males. Diploid male production may effectively curb evolution of sociality, and advanced social behavior in euglossines is positively associated with genetic polymorphism.

Corbiculate apines are a monophyletic group that includes all bees that form colonies (and some of their parasites) and have concave "baskets" on hindlegs for carrying pollen and nesting material (Dressler 1982a; Kimsey 1987; Michener 1990; Prentice 1991; Cameron 1993; Roig-Alsina and Michener 1993). One such group that is strictly Neotropical, the Euglossini or "orchid-bees," had not been studied for population-genetic traits. In addition, among their 200 species the euglossines do not include a single advanced social bee, in contrast to the other corbiculate Apinae—the honey bees, bumblebees, and stingless bees—all of which form colonies with a queen, workers, and stored honey and pollen (Roubik 1989). In the Hymenoptera and some other insects, a few known characteristics, haplodiploidy for instance, appear broadly related to social evolution or level of enzyme polymorphism (Crespi 1991; Packer 1991; Rosenmeier and Packer 1993). Euglossine bees are excellent subjects for study because their males are relatively common and easily collected by using chemical attractants. Many aspects of their biology are known, especially at the generic level (Michener 1974; Ackerman 1983; Roubik 1989). In lowland Panama, we studied euglossine genetic polymorphism and found none in the highly seasonal genus, *Eufriesea,* or the brood parasite, *Exaerete.* However, the colonial genera *Euglossa* and *Eulaema* showed unusually high polymorphism and high proportions of diploid males. These comprised an estimated 12–100% of all males.

Materials and Methods

Nine species in four genera were the primary genetic subjects. Males were collected by multiple baiting runs, making 2–17 collections of 4 h each, over a period of 18 mo (see Roubik and Ackerman 1987). Six more species were used during preliminary screening for variable systems (Appendix, Table 1). After assays of 23 enzymes, we concentrated on five polymorphic systems encoding six presumptive isozyme loci (Table 1, see also Packer and Owen 1992). Bees included the largest and smallest of all euglossines, and the tribe was represented by species from each genus native to Panama, which encompass all but *Aglae,* an Amazonian, monospecific genus of parasite. Allozyme studies incorporated the entire head and thorax for *Euglossa* and *Eufriesea.* Lateral half-sections of these segments were used for the larger-bodied *Exaerete* and *Eulaema.* Samples were homogenized in an equal volume of grinding buffer (0.25 M sucrose, 2% phenoxyethanol) by hand in a chilled porcelain spot plate. Filter paper wicks were placed on the homogenate and then blotted to remove the excess moisture before loading for standard horizontal starch gel electrophoresis (Murphy et al. 1990). The gel buffers and enzymes stained for the principal analysis were hexokinase (TVB, tris versene borate pH 8.5, Selander et al. 1971), isocitrate dehydrogenase (CT, amine citrate pH 6.0, Clayton and Tretiak 1972), malate dehydrogenase (CT), phosphogluconate dehydrogenase (CT), phosphoglucomutase (CT), aspartate aminotransferase (TC, tris citrate, pH 8.0, Selander et al. 1971), aldehyde oxidase (RW, tris citrate pH 8.5, Ridgway et al. 1970), adenylate kinase (TVB), a-glycerophosphate dehydrogenase (TC), glycero-3-phosphate dehydrogenase (TC), glucose phosphate isomerase (CT), homoserine dehydrogenase (TVB), malic enzyme (TC), peptidase (Val-Leu) (RW), phosphofructokinase (TC), superoxide dismutase (CT), diaphorase (RW), glutamate dehydrogenase (TVB), peptidase (Leu-Gly-Gly) (RW), pyruvate kinase (TVB), and triose phosphate isomerase (TC).

Field samples usually consisted of 60 males for the principal species. Sites included cloud forest (Cerro Campana, 800 m), three middle elevation (400–500 m) wet forests (Santa Rita Ridge, El Llano-Carti Road "Nusagandi," Cerro Jefe), and lowland moist forest (Pipeline Road, km 12, and Barro Colorado Island; 200 m elevation). Two species were also taken in far western Pacific Costa Rica (Santa Rosa National Park, one lowland and one upland site). Field-caught bees were induced to torpor by chilling within plastic vials placed in a small cooler with "blue ice." Samples were later frozen at −80°C.

Results and Discussion

Genetic Variation and Diploid Males

Six loci showed allelic variation within species: MDH, PGM, ICD1 and ICD2, PGDH, and HK, at which two to five alleles were detected. Eighteen other systems also showed

TABLE 1. Population-genetic traits found in euglossine bees by horizontal gel electrophoresis.

Species*	Polymorphism	Heterozygous diploid males at given loci	Estimated diploid males ± SD**	Male *N, n*	Mean N ·locus^{-1}
El. meriana	0.33	0.11, 0.54	0.35–0.81 ± 0.15	37, 4; 44, 24	41.5
Eg. championi	0.17	0.0	N.A.	N.A.	12.0
Eg. imperialis	0.33	0.11, 0.18	0.56–0.65 ± 0.17–0.20	62, 7; 63, 11	59.7
Eg. sapphirina	0.33	0.08, 0.04	0.12–0.54 ± 0.27	48, 2; 40, 3	46.2
Eg. tridentata	0.33	0.03, 0.39	0.14–1.00	35, 1; 44, 17	53.2
Eg. cybelia	0.17	0.0	N.A.	N.A.	5.0
El. nigrita	0.17	0.0	N.A.	N.A.	6.2

* Monomorphic species and mean *N* per locus were: *Exaerete frontalis* (56), *Eulaema cingulata* (58.3), *Eufriesea anisochlora* (57.5), *Eufriesea pulchra* (40.5), *Exaerete smaragdina* (1), *Euglossa cybelia* (5), *Euglossa asarophora* (1.8), *Eulaema nigrita* (6.2), *Eulaema speciosa* (4), and *Euglossa deceptrix* (5).
** Estimate of total proportion of males that are diploid, for 2–5 alleles, SD for diallelic loci only, from gene count data (column listing diploid males *N, n*).

variation between species at the presumptive loci but none intraspecifically. Different species often shared no alleles, and some monomorphic systems showed the same allele in all species—DIA, GDH, PEP, PK, and TPI. The relatively large samples for nine species revealed that five had detectable enzyme polymorphism: *Eulaema meriana, Euglossa tridentata, Euglossa imperialis, Euglossa sapphirina,* and *Euglossa championi* (Table 1). Expected mean heterozygosities were 0.07–0.14, with 16.7–33% polymorphism at the five intraspecifically polymorphic isozyme loci. Variability at PGM and HK loci was found also in *Eulaema nigrita* and *Euglossa cybelia.* Reliably scorable banding patterns differed among species, thus samples were from 35–63 haploid genomes, depending on the isoenzyme studied. Only 12 individuals of *Euglossa championi* were sampled but over a considerably larger geographic range (Appendix).

Four species had high frequencies of heterozygous (diploid) males detected by characteristic single-locus banding patterns: two bands (1:1, equal in staining intensity) in monomeric, three (1:2:1 intensity) in dimeric, and five (1:4:6:4:1 intensity) in tetrameric patterns. Gene counts and the range of possible diploid male frequencies and their standard deviations are given in Table 1. *Euglossa sapphirina* had 4% and 8% heterozygotes for HK (4 alleles) and PGM (2 alleles), respectively. *Euglossa tridentata* showed 3% and 39% heterozygous males for HK (3 alleles) and PGM (3 alleles). Male *Euglossa imperialis* were heterozygous at 11% and 18% frequency for PGM and MDH, each showing two alleles. *Eulaema meriana* had 35% heterozygotes for HK (2 alleles) and 54% heterozygosity for PGM (5 alleles). These figures underestimate true diploid male number, because homozygous diploids were not detectable (Owen and Packer 1994; see Table 1).

Some genetic differences were noteworthy. Cloud forest populations of *Eulaema meriana* and *Euglossa championi* were unlike lowland populations at the HK locus. A single *Eulaema nigrita* male from Costa Rica was diploid and possessed two PGM alleles different from *Eulaema nigrita* in Panama. In addition, two species of *Eulaema* that form mixed colonies (*Eulaema polychroma* and *Eulaema cingulata*; see Roubik 1990) were monomorphic for different alleles.

Diploid males have never been found in natural bee populations in proportions as high as those seen in this study (Kukuk and May 1990; Packer and Owen 1990; Duchateau et al. 1994). Moreover, the number of males sampled has often been too low to include rare alleles expressed in diploid males. Thus, direct karyotypic analysis would be a more effective technique. For halictine bees, two samples that included 60 and 125 males produced seven and two diploids (Kukuk and May 1990), and one diploid male occurred in 185 (Packer and Owen 1990). Thus, 10 diploid males accrued among 370. Summarizing Table 1, we found 55 diploid males among 199 in species with enzyme polymorphisms. For allozyme loci to reveal proportions of diploid hymenopteran males in excess of 10%, sample sizes still require 20–100 males, at allele frequencies ranging from 0.5–0.05 (Owen and Packer 1994). Calculated proportions of euglossine diploid males (among all males in the sampled population) ranged from 1.0–0.12 (Table 1). Standard deviations of estimators for diploid male frequency were calculated following procedures given in Owen and Packer (1994) for diallelic systems. A matrix algebra determinant can be used to compute variance in the estimate of diploid male proportion for three or more alleles (Owen and Packer 1994).

We believe that diploid euglossine males have no fitness, at most siring infertile females. Diploid males were not larger than or clearly distinctive from haploid males, and they responded to phytochemical lures as do normal males. Collection of phytochemicals by males is probably a normal prelude to successful courtship (Roubik 1989; Whitten et al. 1989; Armbruster 1993). Homozygosity at the sex locus leads to 50% of diploids being male in Hymenoptera, and diploid males are usually present, at some stage of the life cycle (Crozier 1971; Woyke 1986). A low number of alleles at a sex-determining locus, relative to other corbiculate apines, would lead to more frequent homozygosity and production of diploid males in euglossines, as would inbreeding (Duchateau et al. 1994). Only two alleles may have been present at the sex locus among *Euglossa tridentata* at Pipeline Road (Appendix, Table 1), where one-half of all diploids presumably matured to adult males.

Maximum likelihood estimates of diploid male frequency are based on several assumptions, including that only one offspring per random mating is included in a sample. In our samples, most male bees of some species were taken on one day at some localities (Appendix). As noted in several studies, however (summary in Ackerman et al. 1982), euglossine males may range widely during their life and do not return to nests. Males at one site were usually collected over a period of months or years. Within one day they were collected over

hours, incorporating common species from among hundreds of individuals (Roubik and Ackerman 1987).

Matings rarely occur, even in abundant euglossine species (Stern 1991); thus, male competition and outcrossing enforced by female choice are likely, but no data are available on frequencies of individual male mating. Highly fit males could concievably sire many offspring with different females, and inbreeding could thereby result. Colonies are composed of one or few adults and only several to a few dozen brood (Roubik 1989; Garófalo et al. 1993). For *Euglossa imperialis,* data from two alleles at PGM and MDH were in concordance, suggesting that 54–65% of males were diploid, with a standard deviation of 20%. Most of the males came from a single location, Pipeline Road (Appendix). Estimates for the other species were more variable. At Pipeline Road, from five collections made in 1991 and 1992, both PGM and HK showed three alleles in *Euglossa tridentata,* and estimated proportions of diploid males were 0.14–1.00, although 80% of the males used were the same for both isoenzymes (Table 1). Samples of the 37–45 haploid genomes had standard deviations similar in magnitude to those for diallelic loci. *Euglossa sapphirina,* with two and four alleles expressed in PGM and HK, respectively, yielded estimates of 0.12 (SD 0.27) and 0.54 diploid males. *Eulaema meriana,* with HK and PGM systems showing two and five alleles, respectively, was estimated to have 0.35 (SD 0.15) and 0.81 diploid males. According to Owen and Packer (1994), the sample size needed to produce a heterozygous male is easily calculated as a function of true diploid male proportion and allele frequency at the marker locus. For the four diallelic loci found in our euglossines, frequency of the rare allele (0.1) and diploid males (0.3) suggest that samples of 20 males would be sufficient to reveal diploids. Samples of 53 or more should have been adequate to detect diploid male frequencies of 0.20 or higher for almost all allele frequencies. However, only three other species showed allelic variation. Samples were meager (5, 7, and 12 bees) and, therefore, unlikely to show whether heterozygous males were present in the population. Our conclusion is that all euglossines potentially have high proportions of diploid males. Other hymenopteran populations, including bees, have seldom been sampled adequately to determine whether high proportions of diploid males are uncommon.

Intergeneric Distribution of Social and Other Traits

Our results establish fertile ground for study by showing ample, detectable polymorphism and new paradigms regarding evolution of sociality in bees and the abundance of diploid males in haplodiploid populations. Degree of sociality was inferred from species-group and subgenus in *Euglossa* (Dressler 1978, 1982a,b), because specific data were unavailable for *Euglossa sapphirina, Euglossa cybelia,* and *Euglossa tridentata.* However, colony formation by genera clearly was related to population-genetic characteristics, as were other suggested life-history categories. The more social bees, *Eulaema* and *Euglossa,* were genetically polymorphic (Table 1). Solitary, highly seasonal, or parasitic genera, *Eufriesea* and *Exaerete,* were monomorphic. Berkelhamer (1983) predicted loss of genetic variation as a result of social evolution, but we demonstrate a significant, opposite trend in euglossines. This may be a result of sharp differences in sociality and factors that regulate genetic polymorphism among euglossines. To evaluate a general hypothesis that the more social euglossines are more genetically polymorphic, it would be desirable to sample widely among *Eulaema* and *Euglossa,* and at the same time obtain details on social interactions, now available only for a few species (Zucchi et al. 1969; Garófalo 1985; Eberhard 1988; Garófalo et al. 1993). Rosenmeier and Packer (1993) showed that genetic variation had no association with nesting biology in two distantly related Hymenoptera, and we found no difference in the genetic polymorphism of species using exposed versus protected nesting sites among *Eulaema* and *Euglossa.* However, the same caveat mentioned for social behavior would apply to nesting biology, which embraces not only evolutionary or adaptive potentials but many other details, for example the frequency and kind of predation and parasitism (Roubik 1989, 1990).

Little to moderate polymorphism, averaging $< 10\%$, depending on taxa and techniques, has been found in corbiculate apines—Apini, Meliponini, and Bombini. An average of 17% polymorphic loci has been found for bees that are largely solitary, showing no degree of sociality (Sylvester 1986; Roubik 1989; Packer and Owen 1990, 1992; Contel et al. 1992). Although mean heterozygosity among advanced eusocial Hymenoptera is approximately half that of the solitary and primitively eusocial species, such comparative data lack direct comparison within clades, even at the family level (Packer and Owen 1990). Euglossine polymorphism, at 15% across loci here, is near the average for bees but evidently twice that of bumblebees, stingless bees, and honey bees (Contel et al. 1992; Packer and Owen 1992).

Limits to Social Evolution

Why do euglossines lack the kind of colonies formed by all their close relatives? Significant hindrances to establishing perennial colonies might arise from both high diploid male production and genetic polymorphism. Up to 50% of the intended females (diploids) would be males. Thus, the supply of altruistic workers would be erratic. Colonies could not develop adequately, to then culminate in drone and reproductive female production. In addition, because a diploid male larva lives within a thick, closed, resinous or mud cell, females may not readily eliminate them. Diploid males are presumably of no adaptive value to other bees and amount to a loss of energy and resources. Current discussion is limited by our ignorance of mechanisms that prevent inbreeding in euglossines and ways that successful chemical acquisition by males influences mating success or paternity in local bee populations. Inbreeding is a likely reason for high diploid male proportion. At the same time, a threshold of genetic homogeneity permitting colony fitness to replace individual expected fitness may not have been reached by Euglossini, compared to sister taxa. Lowered polymorphism might be seen as a basis for advanced social evolution, rather than a consequence (Berkelhamer 1983). Levels of euglossine genetic polymorphism among social genera appear even higher than those of ordinary, solitary bees, and much higher than other corbiculate apines (Table 1). Further comparisons seem

promising for seeking explanations of both social evolution and polymorphism, considering euglossine bees.

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APPENDIX.

Principal bee collections for genetic study, and localities.

Species sampled	Locality	No. sampled	No. collections	No. gels
Exaerete frontalis	Pipeline Road	54	11	10
	Barro Colorado Island	5	3	2
	Cerro Campana	2	2	1
	El Llano-Cartí Road	1	1	1
Eulaema meriana	El Llano-Cartí Road	2	2	2
	Pipeline Road	31	4	5
	Cerro Campana	23	2	4
	Cerro Jefe	1	2	1
	Barro Colorado Island	1	3	1
Eulaema cingulata	Cerro Campana	10	2	2
	Pipeline Road	45	1	6
	Madden Highway	5	1	1
Euglossa tridentata	Pipeline Road	65	5	9
Euglossa imperialis	Pipeline Road	61	3	10
	Barro Colorado Island	1	1	1
Euglossa championi	Cerro Campana	5	1	1
	Barro Colorado Island	2	1	1
	Volcan Orosí	5	1	1
Euglossa sapphirina	El-Llano Cartí Road	24	2	2
	Cerro Jefe	5	3	1
	Nusagandi	13	2	2
	Santa Rita Ridge	8	1	2
	Barro Colorado Island	1	1	1
Eufriesea anisochlora	Cerro Campana	30	1	5
	Cerro Jefe	30	1	5
Eufriesea pulchra	Barro Colorado Island	2	1	2
	Pipeline Road	40	8	9

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MORPHOMETRIC DIFFERENTIATION IN SERIALLY BOTTLENECKED POPULATIONS OF THE HOUSEFLY

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A population bottleneck is often invoked in speciation theory as a mechanism to overcome contraints to genetic change inherent in large random-bred populations. However, there is little direct evidence that bottlenecks accelerate genetic change (e.g., see review by Barton and Charlesworth 1984), and to the contrary, Charlesworth and Smith (1982), Rouhani and Barton (1987), and Charlesworth and Rouhani (1988) have argued that bottlenecks may retard rather than enhance a peak shift in a Wrightian landscape. We have provided experimental evidence that populations recovered from a single-pair bottleneck diverged from an ancester preferentially along multivariate axes of morphological shape rather than of size, based on generalized genetic distances from the ancestor (Bryant and Meffert 1990). Populations bottlenecked to lesser extents did not show such disproportionate shape changes, suggesting that only severe bottlenecks may promote changes in morphological shape in the way envisioned in models of speciation (e.g., Carson 1968; Templeton 1980).

Two of the original four replicate lines for each bottleneck size treatment (one, four, or 16 pairs) were subjected to four additional founder-flush cycles. The levels of additive genetic variance deteriorated over the five founder-flush cycles much more rapidly for the single-pair lines than for the four- and 16-pair lines (Bryant and Meffert 1993), and thus these larger bottlenecked lines may have retained ability for continued

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