

Use of diploid male frequency data as an indicator of pollinator decline

Amro Zayed^{1*}, David W. Roubik² and Laurence Packer¹

¹Department of Biology, York University, 4700 Keele Street, Toronto, Ontario M3J 1P3, Canada

²Smithsonian Tropical Research Institute, Box 2072, Balboa, Ancon, Republic of Panama

* Author for correspondence (amro@torontodigital.com).

Recd 10.08.03; Accptd 17.09.03; Online 31.10.03

Pollination deficits in agricultural and natural systems are suggestive of large reductions in pollinator populations. However, actual declines are difficult to demonstrate using census data. Here, we show census data to be misleading because many abundant pollinators exhibit high levels of production of sterile diploid males usually found only in small inbred hymenopteran populations; *Euglossa imperialis* exhibits high levels of diploid male production induced by low effective population sizes ($N_e \approx 15$), despite being the most abundant orchid bee in lowland tropical forests in Panama. We caution that although some pollinators appear abundant on the basis of census data, their long-term persistence may be highly tenuous based on genetic evidence. We propose the use of diploid male frequency data as a metric for assessing the sustainability of bee populations.

Keywords: pollinator decline; diploid males; Euglossini; Hymenoptera

1. INTRODUCTION

Owing to their role in pollination, bees are crucial for the maintenance of terrestrial biodiversity and their conservation is thereby of prime importance (Kearns *et al.* 1998; Kevan 1999). Pollination deficits suggest that bees are undergoing large population reduction (Kevan & Phillips 2001); however, actual pollinator declines have been difficult to demonstrate using census data (Roubik 2001; Williams *et al.* 2001, but see Frankie *et al.* 1997; Kevan *et al.* 1997). Because our ability to conserve pollinators depends on our ability to detect when their populations are at risk, developing accurate parameters to assess pollinator declines is essential. Genetic parameters have the potential to indicate small populations at risk of extirpation (Avise & Hamrick 1996; Petit *et al.* 1998). However, their role in bee conservation remains largely unexplored as standard conservation genetic models are not directly applicable to haplodiploids (Packer & Owen 2001).

One criterion that we show is ideal for detecting loss of genetic diversity in haplodiploids is the production of diploid males (Packer & Owen 2001; Zayed & Packer 2001).

Diploid males arise from fertilized eggs homozygous at the sex determination locus, whereas heterozygotes develop into females, and hemizygotes into haploid males (Cook & Crozier 1995). Through their sterility or inviability (Stouthamer *et al.* 1992; Agoze *et al.* 1994), the production of diploid males is disadvantageous because it increases the genetic load and decreases reproductive fitness (Page 1980; Ross *et al.* 1993) and, in social species, increases colony mortality and decreases colony growth rates (Plowright & Pallett 1979; Ross & Fletcher 1986). However, natural populations usually maintain many alleles at the sex locus and thus diploid males occur at very low frequencies (Cook & Crozier 1995). The number of alleles at the sex locus is maintained at mutation-drift equilibrium, with larger populations maintaining more alleles than smaller ones (Yokoyama & Nei 1979).

A study of Euglossine-orchid bees in Panama revealed that many species have unusually large numbers of diploid males, with frequencies ranging from 12% to 100% (Roubik *et al.* 1996). This is paradoxical considering that censuses indicate that orchid bees exist in large populations (Roubik 2001). High frequencies of diploid males were not found in Brazilian orchid bees (Takahashi *et al.* 2001), prompting our research into the causes of such an unusual phenomenon in apparently healthy Panamanian bee populations. Here, we provide an explanation for this paradox. Through an extensive study of one species, *Euglossa imperialis* Cockerell, the most abundant orchid bee in lowland forest in Panama (Roubik 2001), we confirm that there are indeed high levels of diploid males and that this results from small effective population sizes (N_e). We highlight the factors responsible for creating the large discrepancies between the census population size and N_e in this species, and discuss the utility of data on the production of diploid males as indicators of the long-term viability of pollinator populations.

2. MATERIAL AND METHODS

Euglossa imperialis males were collected using cineole and methyl salicylate baits (Ackerman 1983) during February 2002. Site symbols, names and sample sizes are: (A) Pipeline Road-40; (B) El Llano Carti Road-18; (C) Howard Airforce Base-32; (D) Fort Clayton-21; (E) Barro Colorado Island-93; (F) Cerro Jefe-41; (G) Fort Sherman-60; (H) Madden Dam-11; (I) Cerro Campana-30; (J) Gigante Peninsula-51; (K) Cerro Hoya-94; (L) Cuesta de Piedra-25; (M) Santa Rita Ridge-99; (N) Maria Chiquita-25; and (O) Rio Caimito-55.

Genetic variation was assayed at the allozyme loci esterase (EST), hexokinase (HK) and phosphoglucosmutase (PGM) following Packer & Owen (1990). The frequency of diploid males, ϕ , was estimated for each locus using maximum likelihood (Owen & Packer 1994). The effective number of alleles K was estimated given the expected frequency of haploids in a population h (with a 1 : 1 sex ratio; Peruquetti & Campos 1997) using the equation (derivation to be presented elsewhere) $K = (1 - h)(1 - \phi)/h\phi$. Single-locus estimates of ϕ and K were averaged over loci to obtain population values.

N_e was estimated in two ways, as follows.

- (i) Iteratively by fixing a mutation-drift model (Yokoyama & Nei 1979, eqn (30)) for the frequency of sex locus homozygotes given the average K estimated and assuming a mutation rate of $u = 10^{-5}$.
- (ii) By regressing the log of pairwise geneflow (N_m), estimated from F_{ST} (Wright 1951) against the log of pairwise distance for all population pairs.

N_e is equivalent to 10^b , where b is the y -intercept of the isolation by distance plot (Slatkin 1993). We simulated diploid populations of equal sizes to those sampled based on male allele frequencies to estimate F_{ST} using FSTAT 2.9.3 (Goudet 1995). Average pairwise F_{ST} from 100 simulations was used to estimate N_m .

Rates of heterozygosity loss by drift in ideal isolated populations were estimated following Crow & Kimura (1970). Populations were

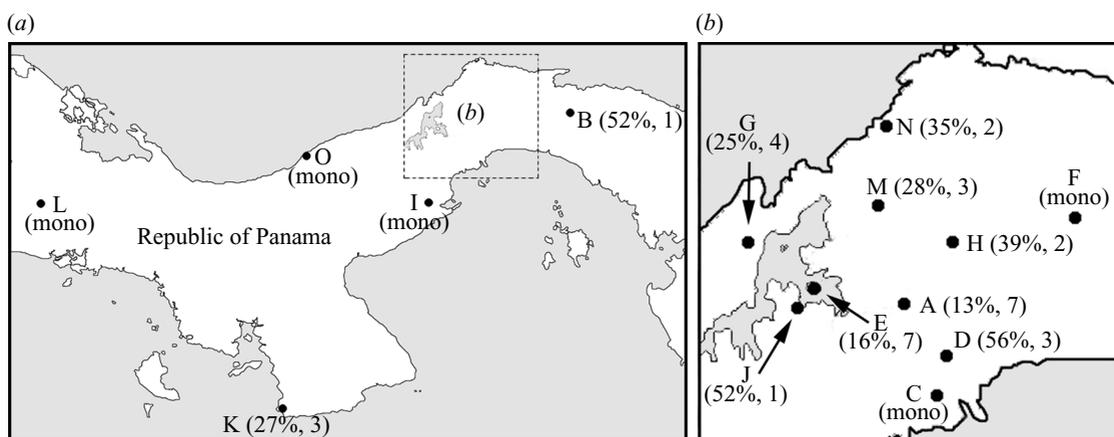


Figure 1. (a) Map of Panama and (b) magnification of boxed section, showing sampling locations, average frequencies of males that are diploid ϕ , and the effective number of sex alleles K for *Euglossa imperialis*. Monomorphic populations are labelled '(mono)'.

classified as monomorphic if the frequency of the most common allele at all loci was greater than 95%.

To estimate the census population size, we used data recorded during May 1980 to May 2000 (Roubik 2001). Because only males are attracted to the baits, the total population size is double the number of males observed, given a 1:1 sex ratio (Peruquetti & Campos 1997).

3. RESULTS

Ten out of the 15 populations sampled were polymorphic, with an average expected heterozygosity of $H_{exp} = 0.257$. The average proportion of males that are diploid ϕ is 34.3% (ranging from 13% to over 50%), with a mean of only 3.1 sex alleles (figure 1). Using the mutation-drift model, we obtained an estimate of $N_e = 23.1$ individuals. The isolation by distance model provided an independent estimate of $N_e = 7.3$ individuals (95% CI = 3.5–17), giving an average for the two estimates of only 15.2 individuals, representing *ca.* 5% of the average censused population size of 295.

4. DISCUSSION

Populations of *E. imperialis* in Panama have unusually high frequencies of diploid males induced by chronically low N_e . Our finding of five monomorphic populations (figure 1), representing a third of the populations sampled, is consistent with low N_e and extreme loss of allelic diversity. Complete loss of heterozygosity from the average of $H_{exp} = 0.257$ found in polymorphic populations, occurs in 50 generations in an isolated population with $N_e = 15.2$, but requires 967 generations given the census population size. How can we reconcile our low estimates of N_e with the high census estimates for *E. imperialis*?

First, population sizes of orchid bees in Panama vary dramatically over time (Roubik 2001) and N_e is bound by the lowest census size in a series of fluctuations (Nunney & Elam 1994). Second, male reproductive success is influenced strongly by the complexity of the chemical bouquets that they obtain from flowers, particularly rare orchids (Roubik 1998; Eltz *et al.* 1999). High variance in reproductive success, combined with overlapping generations (Roubik 1989), considerably reduce N_e with respect to N (Felsenstein 1971; Crow & Denniston 1988). Also, census estimates are based on sampling both the

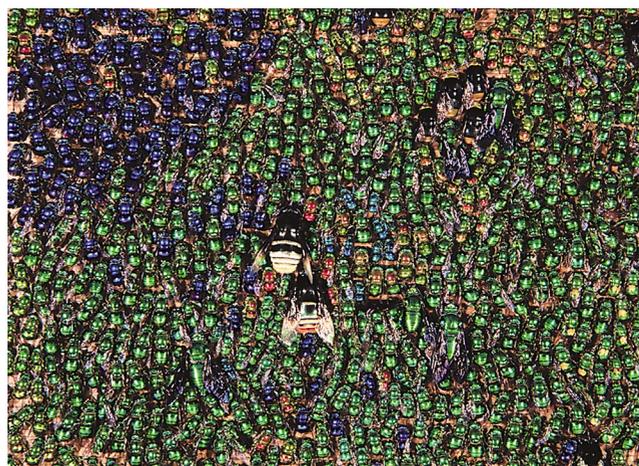


Figure 2. Male orchid bees (species from the genera *Euglossa*, *Eulaema*, *Eufriesea* and *Exaerete* are depicted in the above photograph) are easily censused using chemical baits. However, despite their apparent abundance based on census data, several species exhibit a genetic phenomenon prevalent in small haplodiploid populations: the production of sterile diploid males. Census data obtained from baiting greatly overestimate the effective population size of these important pollinators, and often lack sufficient power to detect pollinator declines. Photograph, D.W.R. and M. Guerra.

haploid and the diploid males attracted to chemical baits (figure 2; Ackerman 1983), but sterile diploid males do not contribute to reproduction (Cook & Crozier 1995). Furthermore, since diploid males arise from fertilized eggs, they are failed attempts at female production, and their presence in large frequencies drains the population of females, which also reduces N_e (Crozier 1976). Taken in combination, these factors suggest why census data overestimate N_e by more than an order of magnitude.

Finally, the dynamics of metapopulation extinction and recolonization, which can reduce the effective population size by several orders of magnitude (Gilpin 1991; McCauley 1993), might be responsible for the low effective population size of *E. imperialis*. Although it is generally believed that Euglossines are capable of long-range dispersal, based on their flight abilities (Roubik 1989), our study shows

that gene flow is geographically restricted. Geographical variance in the number of sex alleles (figure 1) and expected heterozygosity across Panama, are consistent with a metapopulation structure with a few large source populations and many small sink populations.

Orchid bees play a major role in neotropical ecosystems. The males are the sole pollinators of several hundred species of orchids, and females pollinate many trees, shrubs and vines (Ramirez *et al.* 2002). Small N_e , as shown for *E. imperialis*, is probably occurring in other Panamanian orchid bees, several of which have even higher estimates of production of diploid males (Roubik *et al.* 1996). Significantly, many of our sample sites lie in protected forests, some of which are over 10 000 ha in area. The long-term persistence of these bees is unlikely simply on genetic grounds (Saccheri *et al.* 1998; Westemeier *et al.* 1998; Higgins & Lynch 2001), and indeed, *E. imperialis* populations, although apparently large, are declining even in extensively protected forests (Roubik 2001).

Our findings have far-reaching implications for the conservation of the Hymenoptera. First, the frequency of diploid males is a sensitive indicator of genetic diversity and its loss. Baseline data on ϕ and K for native bee species should be compiled to facilitate future population monitoring. Second, the conservation status of hymenopteran populations should be addressed with genetic data, as assessments based solely on census data can be misleading. Additionally, high temporal variability in censused abundance of tropical and temperate pollinators impedes the detection of meaningful trends (Roubik 2001; Williams *et al.* 2001). Genetic parameters such as N_e and ϕ are insensitive to such short-term fluctuations. Further diploid male frequency data can be easily gathered and translated to N_e , making the Hymenoptera a good indicator taxon to assess the current health of ecosystems.

Acknowledgements

The authors thank Mr R. Hartmann for providing accommodation in Santa Clara, Mr B. Boyd for providing access to Rio Caimito, J. Grixti and G. Arbabi for assistance in running gels, J. Young for sharing expertise on iterative functions, and two anonymous referees for helpful comments on the manuscript. This work was supported by an American Orchid Society grant to the authors, a NSERC grant to L.P., and NSERC and OGS scholarships to A.Z.

Ackerman, J. D. 1983 Specificity and mutual dependency of the orchid–euglossine bee interaction. *Biol. J. Linn. Soc.* **20**, 301–314.
 Agoze, M. E., Drezon, J. M., Renalt, S. & Preiquet, G. 1994 Analysis of the reproductive potential of diploid males in the wasp *Diadromus pulchellus* (Hymenoptera: Ichneumonidae). *Bull. Entomol. Res.* **84**, 213–218.
 Avise, J. C. & Hamrick, J. L. (eds) 1996 *Conservation genetics, case histories from nature*. New York: Chapman & Hall.
 Cook, J. M. & Crozier, R. H. 1995 Sex determination and population biology of the Hymenoptera. *Trends Ecol. Evol.* **10**, 281–286.
 Crow, J. F. & Denniston, C. 1988 Inbreeding and variance effective population numbers. *Evolution* **42**, 482–495.
 Crow, J. F. & Kimura, M. 1970 *An introduction to population genetics theory*. New York: Harper & Row.
 Crozier, R. H. 1976 Counter-intuitive property of effective population size. *Nature* **262**, 384.
 Eltz, T., Whitten, W. M., Roubik, D. W. & Linsenmair, K. E. 1999 Fragrance collection, storage, and accumulation by individual male orchid bees. *J. Chem. Ecol.* **25**, 157–176.
 Felsenstein, J. 1971 Inbreeding and variance effective numbers in populations with overlapping generations. *Genetics* **68**, 581–597.

Frankie, G. W., Vinson, S. B., Rizzardi, M. A., Grisold, T. L., O'Keefe, S. & Snelling, R. R. 1997 Diversity and abundance of bees visiting a mass flowering tree species in disturbed seasonal dry forest, Costa Rica. *J. Kansas Entomol. Soc.* **70**, 281–296.
 Gilpin, M. E. 1991 The genetic effective size of a metapopulation. *Biol. J. Linn. Soc.* **42**, 165–175.
 Goudet, J. 1995 FSTAT (vers. 1.2): a computer program to calculate F-statistics. *J. Heredity* **86**, 485–486.
 Higgins, K. & Lynch, M. 2001 Metapopulation extinction caused by mutation accumulation. *Proc. Natl Acad. Sci. USA* **98**, 2928–2933.
 Kearns, C. A., Inouye, D. W. & Waser, N. M. 1998 Endangered mutualisms: the conservation of plant–pollinator interactions. *A. Rev. Ecol. Syst.* **29**, 83–112.
 Kevan, P. G. 1999 Pollinators as bioindicators of the state of the environment: species, activity and diversity. *Agric. Ecosyst. Environ.* **74**, 373–393.
 Kevan, P. G. & Phillips, T. P. 2001 The economic impacts of pollinator declines: an approach to assessing the consequences. *Conserv. Ecol.* **5**, 8. See <http://www.consecol.org/vol5/iss1/art8>.
 Kevan, P. G., Greco, C. F. & Belaoussoff, S. 1997 Log-normality of biodiversity and abundance in diagnosis and measuring ecosystemic health: pesticide stress on pollinators on blueberry heaths. *J. Appl. Ecol.* **34**, 1122–1136.
 McCauley, D. E. 1993 Genetic consequences of extinction and recolonization in fragmented habitats. In *Biotic interactions and global change* (ed. P. M. Kareiva, J. G. Kingsolver & R. B. Huey), pp. 217–233. Sunderland, MA: Sinauer.
 Nunney, L. & Elam, D. R. 1994 Estimating the effective population size of conserved populations. *Conserv. Biol.* **8**, 175–184.
 Owen, R. E. & Packer, L. 1994 Estimation of the proportion of diploid males in populations of Hymenoptera. *Heredity* **72**, 219–227.
 Packer, L. & Owen, R. 1990 Allozyme variation, linkage disequilibrium and diploid male production in a primitively social bee *Augochlorella striata* (Hymenoptera: Halictidae). *Heredity* **65**, 241–248.
 Packer, L. & Owen, R. 2001 Population genetic aspects of pollinator decline. *Conserv. Ecol.* **5**, article 4. See <http://www.consecol.org/vol5/iss1/art4>.
 Page, R. E. 1980 The evolution of multiple mating behavior by honey bee queens (*Apis mellifera*). *Genetics* **96**, 263–273.
 Peruquetti, R. C. & Campos, L. A. O. 1997 Aspectos da biologia de *Eufriesea violacea* (Blanchard) (Hymenoptera, Apidae, Euglossini). *Rev. Bras. Zool.* **14**, 91–97.
 Petit, R. J., Mousadik, A. E. & Pons, O. 1998 Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.* **12**, 844–855.
 Plowright, R. C. & Pallett, M. J. 1979 Worker-male conflict and inbreeding in bumble bees (Hymenoptera: Apidae). *Can. Entomol.* **111**, 289–294.
 Ramirez, S., Dressler, R. L. & Ospina, M. 2002 Abejas euglossinas (Hymenoptera: Apidae) de la Región Neotropical: Listado de especies con notas sobre su biología. *Biota Colombiana* **3**, 7–118.
 Ross, K. G. & Fletcher, D. J. C. 1986 Diploid male production—a significant colony mortality factor in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **19**, 283–291.
 Ross, K. G., Vargo, E. L., Keller, L. & Trager, J. C. 1993 Effect of a founder event on variation in the genetic sex-determining system of the fire ant *Salenopsis invicta*. *Genetics* **135**, 843–854.
 Roubik, D. W. 1989 *Ecology and natural history of tropical bees*. Cambridge University Press.
 Roubik, D. W. 1998 Grave-robbing by male *Eulaema* (Hymenoptera, Apidae): implications for euglossine biology. *J. Kansas Entomol. Soc.* **71**, 188–191.
 Roubik, D. W. 2001 Ups and downs in pollinator populations: when is there a decline? *Conserv. Ecol.* **5**, article 2. See <http://www.consecol.org/vol5/iss1/art2>.
 Roubik, D. W., Weigt, L. A. & Bonilla, M. A. 1996 Population genetics, diploid males, and limits to social evolution of euglossine bees. *Evolution* **50**, 931–935.
 Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. & Hanski, I. 1998 Inbreeding and extinction in a butterfly metapopulation. *Nature* **392**, 491–494.
 Slatkin, M. 1993 Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**, 264–279.
 Stouthamer, R., Luck, R. F. & Werren, J. H. 1992 Genetics of sex determination and the improvement of biological control using parasitoids. *Environ. Entomol.* **21**, 427–435.

- Takahashi, N. C., Peruquetti, R. C., Del Lama, M. A. & de Oliveira Campos, L. A. 2001 A reanalysis of diploid male frequencies in euglossine bees (Hymenoptera: Apidae). *Evolution* **55**, 1897–1899.
- Westemeier, R. L., Brawn, J. D., Simpson, S. A., Esker, T. L., Jansen, R. W., Walk, J. W., Kershner, E. L., Bouzat, J. L. & Paige, K. N. 1998 Tracking the long-term decline and recovery of an isolated population. *Science* **282**, 1695–1698.
- Williams, N., Minckley, R. & Silveira, F. 2001 Variation in native bee faunas and its implications for detecting community changes. *Conserv. Ecol.* **5**, article 7. See <http://www.consecol.org/vol5/iss1/art7>.
- Wright, S. 1951 The genetical structure of populations. *Ann. Eugenics* **15**, 323–354.
- Yokoyama, S. & Nei, M. 1979 Population dynamics of sex-determining alleles in honey bees and self-incompatibility alleles in plants. *Genetics* **91**, 609–626.
- Zayed, A. & Packer, L. 2001 High levels of diploid male production in a primitively eusocial bee (Hymenoptera: Halictidae). *Heredity* **87**, 631–636.