High levels of diploid male production in a primitively eusocial bee (Hymenoptera: Halictidae)

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Under single locus complementary sex determination (sl-CSD), diploid males are produced from fertilized eggs that are homozygous at the sex-determining locus. Diploid males are effectively sterile, and thus their production generates a costly genetic load. Using allozyme electrophoresis, a large number of diploid males were detected in natural populations of the primitively eusocial bee, *Halictus poeyi* Lepeletier collected in southern and central Florida during May 2000. Estimates for the proportion of diploids that are male ranged from 9.1% to 50%, while the frequency of matched matings ranged from 18.2% to 100%. The effective number of alleles at the sex-determining locus ranged from two to 11, with an average of five alleles. The effective population size of *Halictus poeyi* was estimated to be 19.6 ± 2.5 SE. These data are interpreted in the light of the biogeographic history of Florida and the social biology/population dynamics of *H. poeyi*.

Keywords: diploid males production, effective population size, *Halictus poeyi*, Hymenoptera, sex determination.

Introduction

Studies on diploid male production in the haplodiploid Hymenoptera suggest that complementary sex determination (CSD) is the ancestral condition of the taxon (Cook, 1993; Cook & Crozier, 1995; Butcher *et al.*, 2000a,b). In single locus (sl) CSD, sex is determined by the complementary action of codominant alleles at a sex-determining autosomal locus (Crozier, 1971; Bull, 1981). Under sl-CSD, heterozygotes at the sex locus develop into diploid females, hemizygotes develop into haploid males, and homozygotes develop into diploid males (Whiting, 1939). Because diploid males are effectively sterile (Stouthamer *et al.*, 1992; Agoze *et al.*, 1994), their production increases the genetic load, and reduces the reproductive success of their parents (Page, 1980; Ratnieks, 1990; Ross *et al.*, 1993). In social Hymenoptera, diploid male production has been shown to increase colony mortality (Ross & Fletcher, 1986), and decrease colony growth rates (Plowright & Pallett, 1979).

There is a finite number of sex alleles in any given population, and therefore a small number of diploid males is expected to be produced by the chance mating of parents sharing a sex allele in common. Such matings are referred to as matched matings (Adams *et al.*, 1977). Assuming females mate with only one male, matched matings will result in 50% of the total diploid offspring being diploid males. In a sl-CSD system, the frequency of matched matings, θ, is expected to arise with a frequency of $2/K$, where K is the effective number of alleles at the sex-determining locus (Adams *et al.*, 1977). Thus, allelic diversity plays a critical role in controlling diploid male production. At equilibrium, K is in mutation–drift balance and increases with population size (Yokoyama & Nei, 1979). Other factors being equal, smaller populations are expected to have a lower diversity of sex alleles and, consequently, higher levels of diploid male production than larger populations. Inbreeding will also increase the frequency of matched matings and, hence, the production of diploid males in a population. This phenomenon is well documented through laboratory inbreeding, which results in the production of diploid males in hymenopteran species exhibiting CSD (e.g. Plowright & Pallett, 1979; Agoze *et al.*, 1994; Heimpel *et al.*, 1999; Butcher *et al.*, 2000a). The frequency of diploid males is thus an important parameter in hymenopteran conservation genetics, as high frequencies may indicate genetic impoverishment resulting from inbreeding, population fragmentation, loss of allelic diversity, or a combination of these factors (Packer & Owen, 2001).

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Two parameters can be used to quantify diploid male production in a population. (i) The proportion of diploids that are male, Φ: defined as the total number of diploid males divided by the total number of diploid individuals (male and female), it is equivalent to $(1 - s)(\theta/2)$ (Adams *et al.*, 1977), where s is the selection coefficient against the production of diploid males. (ii) The proportion of males that are diploid, ϕ: defined as the number of diploid males divided by the total number of males (diploid and haploid) (Owen & Packer, 1994). ϕ is dependent on the frequency of matched matings and on the ratio of fertilized to unfertilized eggs (Owen & Packer, 1994). Therefore, in colonies of social Hymenoptera, ϕ is expected to achieve its highest value when queens are producing workers and not haploid sons. Estimates of Φ and ϕ in natural populations usually lie below 10% (e.g. Kukuk & May, 1990; Packer & Owen, 1990). Unusually high diploid male estimates were recorded for several euglossine bees in Panama (Roubik *et al.*, 1996), and in introduced populations of the fire ant *Solenopsis invicta* in the USA (Ross *et al.*, 1993). Loss of allelic diversity at the sex locus, owing to a founder event, is responsible for high levels of diploid male production in *Solenopsis invicta* in its introduced range (Ross *et al.*, 1993). Causes for high levels of diploid male production in the euglossine bees remain uninvestigated.

Halictus poeyi (Lep.) is a primitively eusocial bee found in SE U.S.A. (Packer, 1999). Morphologically it is indistinguishable from its genetically divergent sibling species *H. ligatus* (Carman & Packer, 1996; Danforth *et al.*, 1998), which is found to the north and west of the range of *H. poeyi*. The two species are sympatric only in the Piedmont along the southeastern edge of the Appalachian mountains. The present contribution documents high proportions of diploid males in samples of *H. poeyi* from Florida and presents possible explanations for this unexpected result based upon *H. poeyi*'s social biology and population dynamics.

Materials and methods

Collection of samples

Halictus poeyi samples were collected from 12 sites in Florida, U.S.A., between 6 and 21 May 2000 (Fig. 1). All bees were collected while foraging mainly on the composite flower *Bidens pilosa* between 10.00 and 15.00 hours, a period of peak bee activity. In the field, bees were killed and stored in a liquid nitrogen container, and later transferred to a −80°C deep freezer at York University.

Allozyme electrophoresis and diploid male detection

Allozyme electrophoresis on horizontal starch gels was used to examine genetic variation and detect diploid

MIL(15♀,0♂)

CRO
(24♀,0♂)

AUG
(30♀,1♂)

POR(29♀,2♂)

OCA
(19♀,2♂)

GUL
(28♀,2♂)

CHR(25♀,0♂)

FOR
(10♀,9♂)

Legend
MIL - Milton
POR - Port Saint Joe
AUG - St. Augustine
CRO - Cross City
OCA - Ocala
GUL - Gulf Hammock
CHR - Christmas
FOR - Fort Pierce
LOR - Lorida
FRU - Fruitville
JUP - Jupiter
KEY - Key West

FRU
(19♀,1♂)

LOR
(24♀,8♂)
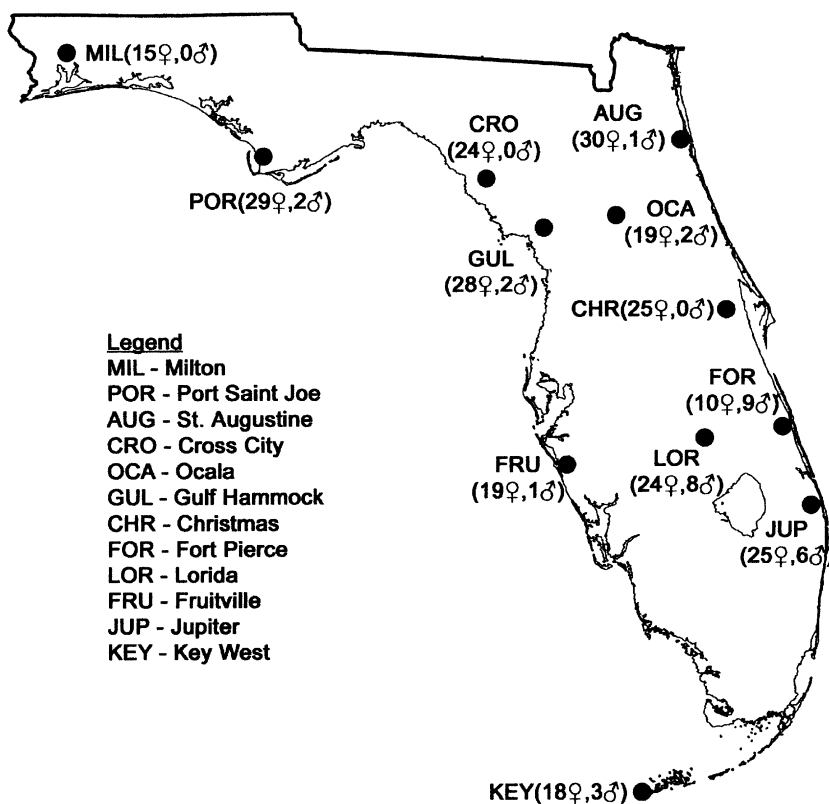
JUP
(25♀,6♂)

KEY(18♀,3♂)

Fig. 1 Map of *Halictus poeyi* study sites in Florida, May 2000. Sample sizes are displayed between brackets.

males in *H. poeyi* samples. Enzyme staining recipes, gel recipes, gel running conditions, and scoring procedures followed Packer & Owen (1989, 1990). Males heterozygous at any locus were considered diploid. Diploid males were detected using six out of the eight polymorphic loci surveyed (Table 1).

Diploid male estimation

Because bees were sampled during the early stages of the colony cycle, where they are mostly producing workers, only Φ, the proportion of diploids that are male, can be estimated with a high degree of confidence. Single locus estimates for the proportion of diploids that are male, Φ, were calculated using the following equation (Owen & Packer, 1994):

$$\hat{\Phi} = \frac{B}{B+A}, \tag{1}$$

where B is the total number of heterozygous males and A is the total number of heterozygous females per locus per sample. The frequency of matched matings θ and the effective number of alleles at the sex determining locus K, assuming sl-CSD, were estimated as follows (Adams *et al.*, 1977; Owen & Packer, 1994):

$$\hat{\theta} = \frac{2\hat{\Phi}}{1-s}, \tag{2}$$

$$\hat{K} = \frac{2}{\hat{\theta}}, \tag{3}$$

Because the selection coefficient against diploid male s is unknown and assumed to be zero, these will be the minimum estimates of θ and K (Owen & Packer, 1994).

Effective population size

The mean effective population size, N_e, was estimated using an isolation-by-distance plot (Wright, 1943; Slatkin, 1993). This was obtained by linear regression of

the log of gene flow vs. the log of distance between site pairs in Florida. Gene flow was assessed as the number of migrants per generation (N_m) using the following equation (Wright, 1951):

$$N_m = \frac{1}{4}\left(\frac{1}{F_{ST}}-1\right), \tag{4}$$

where F_{ST} is the population subdivision statistic. Weir & Cockerham's (1984) estimator of F_{ST} was calculated from the allozyme data using the population genetics software package GENEPOP v.3.2a (Raymond & Rousset, 1995). From the isolation-by-distance plot, the mean effective population size N_e is equivalent to 10^b, where b is the y-intercept of the regression line (i.e. the effective population size is equivalent to log gene flow where log distance is zero). This is also known as the neighbourhood size (Slatkin, 1993).

Results

Diploid male estimates

Diploid males were detected in five out of the 12 sites sampled in Florida (Table 2). From a total of 27 males collected in these five sites, 13 were detected to be diploid using allozyme electrophoresis. Diploid males were detected at the loci shown in Table 2.

Single locus estimates for the proportion of diploids that are male were surprisingly high across all loci and sites (Table 2). They ranged from 9.1% (JUP site-*Alddh*), to 50% (KEY site-*Dia*, etc.), and averaged 29% over all loci and sites. The frequency of matched matings ranged from 18.2% to 100%, and averaged 58%. The number of effective alleles at the sex determining locus ranged from two to 11, and averaged five alleles across all loci and sites.

Effective population size

The isolation-by distance plot (Fig. 2) exhibited a typical pattern of decreasing gene flow over increasing

Table 1 Enzyme names, abbreviations, EC numbers and buffer systems used

Enzyme	Acronym	EC number	Buffer system
Adenylate kinase*	*Ak-1*	2.7.4.3	CAM
Aldehyde dehydrogenase*	*Alddh*	4.1.2.13	V
Arginine kinase*	*Ark*	2.7.3.3	CAM
Diaphorase (NADH)*	*Dia*	1.8.1.n	V
Esterase	*Est-3*	3.1.1.1	RSL
Glucose-6-phosphate dehydrogenase*	*G6pdh*	1.1.1.49	CAM
Hexokinase*	*Hk*	2.7.1.1	I
Phosphogluconate dehydrogenase	*6Pgd-1*	1.1.1.43	CAM

*Indicates enzymes that detected at least one diploid male.

Site	Diploid/total ↵	Locus	Φ (± SD)	θ	K
FOR	3/9	*Ak-1*	0.333 ± 0.272	0.667	3
		Ark	0.333 ± 0.272	0.667	3
		G6pdh	0.500 ± 0.354	1	2
		Hk	0.500 ± 0.354	1	2
LOR	4/8	*Alddh*	0.231 ± 0.117	0.462	4
		Ark	0.500 ± 0.354	1	2
		Dia	0.250 ± 0.217	0.500	4
FRU	1/1	*Alddh*	0.143 ± 0.132	0.286	7
JUP	3/6	*Alddh*	0.091 ± 0.087	0.182	11
		Dia	0.333 ± 0.272	0.667	3
		G6pdh	0.143 ± 0.132	0.286	7
KEY	2/3	*Alddh*	0.125 ± 0.117	0.250	8
		Dia	0.500 ± 0.354	1	2
		Hk	0.167 ± 0.152	0.333	6
Range (%)			9.1–50	18.2–100	2–11
Average (%)			29	58	5

Table 2 Single locus estimates of the proportion of diploids that are male (Φ), the frequency of matched matings (θ), and the effective number of alleles at the sex determining locus (K), for sites that contained diploid males

geographical distance. The regression has a significant *y*-intercept ($t = 3.201209$, $P = 0.0034$) of $1.29 ± 0.40$ (SE), equivalent to a mean effective population size of $19.6 ± 2.5$ (SE) individuals.

Discussion

Diploid male production is a by-product of CSD. Because diploid males are effectively sterile, their production is disadvantageous (Page, 1980; Agoze *et al.*, 1994; Ratnieks, 1990 Stouthamer *et al.*, 1992), and thus they are not expected to occur at a high frequency in natural populations. Most studies coroborate this expectation (Kukuk & May, 1990; Packer & Owen, 1990; reviewed by Cook, 1993). Nonetheless, several researchers have found diploid males at high frequencies. Roubik *et al.* (1996) found that the proportion of males that

Fig. 2 Linear regression between the logarithm of migrants per generation (Nm) and the logarithm of distance (D) for *Halictus poeyi* in Florida, 2000.

were diploid, ϕ, ranged from 12 to 100% in their samples of euglossine bees in Panama. The causes for such high levels of diploid male production awaits further empirical investigations. Unfortunately, as females of these orchid bees are not attracted to chemical baits as the males are, it was not possible for Roubik *et al.* to calculate the proportion of diploids that are male, the statistic required in order to estimate the frequency of matched matings and the effective number of sex determining alleles. Ross *et al.* (1993) also discovered unusually high levels of diploid male production in introduced populations of the fire ant *Solenopsis invicta* in the U.S.A., with ϕ ranging from 73.3 to 100%. This was attributed to the loss of allelic diversity at the sex-determining locus during a recent founder event that led to the invasion of the fire ant in the U.S.A.

High proportions of diploid males were found in populations of *Halictus poeyi* from Florida, where it is a common and widespread species. This combination of high levels of diploid male production in an apparently abundant bee is somewhat enigmatic and requires explanation. There are four likely factors that contribute to this paradox.

1 Like many ground-nesting bees, *H. poeyi* prefers sparsely vegetated nonsandy soil, and such early successional stage habitats are uncommon and temporary, particularly in warm, damp Florida. It is well known that frequent extinction and recolonization events have a highly negative influence on levels of genetic variability (Giplin, 1991), and can reduce the effective population size by several orders of magnitude (Maruyama & Kimura, 1980; McCauley, 1993).
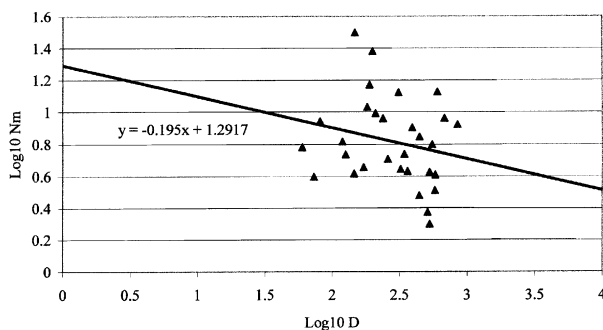
2 Florida has been influenced by repeated sea-level fluctuations, which have, at times, flooded much of the state, causing repeated fragmentation and isolation of animal and plant populations (e.g. Ellsworth *et al.*, 1994; references therein). When combined with the extinction/recolonization population ecology of this species, this would further reduce the effective population size and opportunity for maintenance of large numbers of sex determining alleles.

3 The large-scale habitat destruction resulting from extensive agriculture and the associated use of pesticides will also have resulted in the reduction and fragmentation of populations since European settlement over 200 hundred years ago.

These three factors all suggest that *H. poeyi* has a population structure in Florida which is likely to result in low allelic diversity at the sex locus. One other aspect of its biology suggests that its effective population size may not be as high as it might appear in the field.

4 As a social bee that can build up reasonably large colony sizes from solitarily initiated nests (Packer & Knerer, 1986), *H. poeyi* will often appear far more abundant than its effective population size would suggest as a result of the large number of workers per nest.

Taken together, these factors suggest that the estimated effective population size obtained herein for this species – in the order of around 20 individuals – is not necessarily surprising even though the species can be quite abundant. What is more, because of the aforementioned reasons, such small effective populations may have been in existence for a long period of time.

The calculated average of only five alleles at the sex locus in *H. poeyi* is not so surprising in light of the factors noted above. Although the number of alleles at the sex locus in natural hymenopteran populations usually ranges between nine and 20 (reviewed by Cook & Crozier, 1995), two other studies have found similarly low diversity. Heimpel *et al.* (1999) estimated there to be six sex alleles in a very small population of the parasitoid wasp *Bracon hebetor*. Similarly Butcher *et al.* (2000a,b) found three to four sex alleles for the parasitic wasps *Diadegma fabricianae* and *D. armillata* in small and isolated populations near the northern edge of their natural range.

The existence of diploid males in nature poses an additional problem to the conservation genetics of haplodiploid insects such as the parasitoid wasps and the bees (Packer & Owen, 2001), two groups of considerable importance for biological control and pollination, respectively. Yet there has been little attention paid to this subject. The high proportion of males found to be diploid in a common, primitively eusocial bee suggests a simple means of gathering more data on this topic. Primitively eusocial species with an annual colony cycle, such as almost all social Halictidae, bumblebees and yellowjackets, produce diploids for much of the colony cycle, switching to male and reproductive female production late in the summer (e.g. Packer & Knerer, 1987). Thus, it is predicted that many of the males found during the early summer in these species should be diploid, and if their frequency as a proportion of all diploids can be estimated without bias (e.g. through censuses of brood in nests), accurate measurements of the allelic diversity at the sex locus could be obtained.

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