Detection of diploid males and estimation of sex determination system in the parasitic wasp *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae) using an allozyme as a genetic marker

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Abstract

The presence of diploid males and a sex determination system in *Diadegma semiclausum*, a larval parasitoid of *Plutella xylostella*, was examined by using an allozyme as a genetic marker. Among six enzymes investigated, only phosphoglucosmutase (PGM) was apparently polymorphic. Two discrete bands were detected on electrophoretic banding patterns of PGM, indicating PGM was a monomer with two alleles on one locus. After establishing pure lines of each PGM allele, the heterozygous females were allowed to mate with their sons to examine the occurrence rate of diploid males in their progenies. Thus the numbers of diploid males identified did not differ significantly from those expected from the single-locus complementary sex determination (CSD). Inbreeding and the subsequent emergence of diploid males could partly account for the male biased sex ratio in the laboratory culture of *D. semiclausum*.

Key words: *Diadegma semiclausum*, *Plutella xylostella*, sex determination, diploid male, allozyme variation

INTRODUCTION

Sex determination in Hymenopteran insects is of a haplodiploid type in which females are diploid, being produced from fertilized eggs, and males are haploid produced parthenogenetically from unfertilized eggs. However, the presence of diploid males has been reported in many wasp species since Whiting and Whiting (1925). Cross experiments have shown that diploid males may arise from fertilized eggs when mother wasp mates with a son. This phenomenon allows us to examine the sex determination system in Hymenoptera. Four types of model, i.e., complementary sex determination (CSD; Whiting, 1943), the genic balance model (Cunha and Kerr, 1957), the nucleo-cytoplasmic balance model (Crozier, 1971), and genomic imprinting sex determination (GISD; Poiré et al., 1993; Beukeboom, 1995), have been proposed for Hymenopteran sex determination. In Ichneumonidae, thus far only the single-locus CSD has been demonstrated (Stouthamer et al., 1992; Cook, 1993). In this CSD system, half of all fertilized eggs are expected to generate diploid males under the condition that the female mates with a son.

D. semiclausum (Hellén) is a solitary larval parasitoid of the diamondback moth *Plutella xylostella* (L.). This parasitoid was introduced to New Zealand from England to control *P. xylostella* in 1936 (Fitton and Walker, 1992), and thereafter introduced and released in many East Asian countries (Waterhouse, 1992). The performance of this parasitoid to control *P. xylostella* is thought to be high (Talekar and Yang, 1993). However, provided that the single-locus CSD is applied to *D. semiclausum*, the introduction of a small parasitoid population may result in frequent sibmating and a high proportion of diploid males. Also, male biased sex ratio of the parasitoid when reared at high temperature ($>30^{\circ}\text{C}$) has been reported (Yang et al., 1993), and this may obstruct their availability in the hot summer season. Thus, to consider why more males of *D. semiclausum* are produced in culture, it is important to clarify the sex determination system of the parasitoid. In this study, the author attempted to examine the presence of diploid males and to define the sex determination mechanism of *D. semiclausum* by using allozyme variation as a genetic marker.

MATERIALS AND METHODS

Parasitoid and host. *D. semiclausum* used in this study were introduced from the Asian Vegetable Research and Development Center in Taiwan in 1989. A laboratory culture of *P. xylostella* was originated from a population collected in Mie Prefecture. Adults of *P. xylostella* were put in a glass jar (7.5 cm in diam. $\times$ 13 cm in depth) with a paper lid on which they were allowed to deposit eggs. Eggs on the paper lid were collected 3 days after setting. Larvae were reared on radish sprout in a small plastic cup (9 $\times$ 5 cm). To maintain the parasitoid culture, ca. 200 host larvae were presented to 10–15 female parasitoids in a plastic container (30 $\times$ 20 $\times$ 10 cm) for one day. After parasitism females were removed and some fresh radish sprouts were supplied as food. Rearings were conducted under 20°C, 16L : 8D condition. Newly emerged adults were kept in a plastic container (30 $\times$ 20 $\times$ 10 cm) with 5% honey solution soaked in filter paper as food at 15°C, 16L : 8D. Unless otherwise indicated, 5- to 10-day-old parasitoid females and 3rd-stadium host larvae were used for experiments.

Genetic marker. To detect allozyme variation as a genetic marker, the following six enzymes were investigated using polyacrylamide gel electrophoresis (PAGE): alcohol dehydrogenase (ADH, EC 1.1.1.1), esterase (EST), fumarase (FUM, EC 4.2.1.2), malic enzyme (ME, EC 1.1.1.40), glucosephosphate isomerase (GPI, EC 5.3.1.9), and phosphoglucumutase (PGM, EC 5.4.2.2). The electrophoresis was done by using native minislab gels (75 mm $\times$ 60 mm, 0.75 mm in thickness; 7.5% polyacrylamide resolving gel and 3% polyacrylamide stacking gel). The gels were stained following May (1992). Among the above mentioned enzymes, polymorphism in PGM was detected on electrophoretic banding patterns (Fig. 1A). A simple Mendelian inheritance pattern with allelic codominance was confirmed for the PGM locus (Noda, unpublished data). Pure lines with only S (PGM-S) or F (PGM-F) band were established by crossing for the subsequent experiments (Fig. 1B).

Cross experiment. The procedure for cross

Fig. 1. Electrophoretic banding patterns of PGM in *D. semiclausum*. A: PGM phenotypes of individuals taken randomly from a culture; B: PGM phenotypes of pure lines.

experiments is demonstrated in Fig. 2. PGM-S females (Ps phenotype shown in Fig. 1, *PsPs* genotype) were paired with PGM-F males (Pf phenotype shown in Fig. 1, *Pf* genotype), then allowed to lay eggs into host larvae. Parasitized hosts were reared on radish sprout until parasitoid cocoons formed. These cocoons were kept separately until parasitoids emerged. All emerged F_1 females ought to be heterozygous (*Pfs* phenotype shown in Fig. 1, *PfPs* genotype). Six virgin F_1 females were allowed to lay eggs into 10–20 host larvae separately. Parasitized hosts were reared on radish sprout and one of the emerged males was paired with his mother in a small plastic cup. From 2 days after pairing, females were allowed to lay as many eggs as possible into host larvae every day until death. PGM phenotype of all offspring were checked by PAGE.

RESULTS AND DISCUSSION

Given that the sex determination of *D. semiclausum* follows the single-locus CSD, females ought to have 2 different sex alleles (Fig. 2). Therefore, sons must have one of the maternal sex alleles, and the sex ratio of diploid individuals from a mother-son crossing should be 1:1, assuming that their mortalities are the same. Considering PGM phenotype, combinations of PGM phenotype and sex of diploid individuals should be *Pfs* ♀ : *Pf* or *Ps* ♀ : *Pfs* ♂ : *Pf* or

Fig. 2. The procedure of cross experiment to detect diploid males in *D. semiclausum*.Table 1. Detection of diploid males of *Diadegma semiclausum* by allozyme variation of PGM

Female No.	No. of hosts parasitized	% Pupation	No. of offspring with phenotype ^a				Chi-square test among phenotypes ^c
			FS ♀	F or S ♀	FS ♂	F or S ♂ ^b	
1	116	56.9	3	3	9	(51)	$p > 0.05$ ($\chi^2 = 4.80$)
2	147	81.6	21	10	19	(70)	$p > 0.05$ ($\chi^2 = 4.12$)
3	75	60.0	8	8	10	(19)	$p > 0.05$ ($\chi^2 = 0.31$)
4	53	56.6	6	3	3	(18)	$p > 0.05$ ($\chi^2 = 1.50$)
5	56	58.9	5	4	7	(17)	$p > 0.05$ ($\chi^2 = 0.88$)
6	74	71.6	8	9	7	(29)	$p > 0.05$ ($\chi^2 = 0.25$)

^a F: F band alone, S: S band alone, FS: both F and S bands were detected.^b Including diploid males of PGM homozygote.^c Test for goodness of fit with null hypothesis of FS ♀ : F or S ♀ : FS ♂ = 1 : 1 : 1.

$Ps \text{ } \text{♂} = 1 : 1 : 1 : 1$ according to Mendel's laws. Among these, the electrophoretic banding pattern of Pf and $Ps \text{ } \text{♂}$ is indistinguishable from those of arrhenotokous males with Pf and Ps genotype. Accordingly, numbers of emerged individuals with phenotypes of $Pfs \text{ } \text{♀}$, Pf or $Ps \text{ } \text{♀}$, and $Pfs \text{ } \text{♂}$ were compared to the null hypothesis of 1 : 1 : 1. The results showed that the null hypothesis could not be discarded in terms of a chi-square test (Table 1, $p > 0.05$). Therefore, it appears that the sex determination of *D. semiclausum* follows the single-locus CSD. The other three models, genic balance model, nucleo-cytoplasmic model, and genomic imprinting sex determination model demonstrated in *Nasonia vitripennis* (Dobson and Tanouye, 1998) can not account for the pres-

ence of diploid males with the predicted ratio in mother-son crossings.

Single-locus CSD has been demonstrated in many wasp species. Cook (1993) reviewed sex determination in the Hymenoptera, and showed that single-locus CSD has occurred in at least 3 superfamilies, i.e., Apoidea, Vespoidea, and Ichneumonoidea. In Ichneumonoidea, for example, Speicher and Speicher (1940) reported the presence of diploid males in the braconid *Bracon brevicornis* and demonstrated a single-locus CSD using recessive wing mutants. Hedderwick et al. (1985) demonstrated the occurrence of diploid males of the ichneumonid *Diadromus pulchellus* in an allozyme study and cytological analysis. Detection of single-locus CSD in *D. semiclausum* strengthens the

proposal that this system prevails in Ichneumonidae.

Diploid males may suffer a higher mortality than diploid females and may be sterile. Petters and Mettus (1980) reported the decreased hatchability and possible early larval survival of diploid males in *Bracon hebetor*. Bostian (1934) reported that about 90% of diploid males that survived to adulthood were sterile in *B. hebetor*. Also in *D. pulchellus*, a great number of abnormal spermatozoa were observed in diploid males, and these spermatozoa with degenerative structures were probably not implicated in egg fertilization (Chauvin et al., 1988). In *D. semiclausum*, mortality in diploid males may not differ from that in diploid females, because the numbers of diploid males emerged were not significantly different from diploid females (Table 1). Since diploid males of *D. semiclausum* are not distinguishable by external characters, it is necessary to identify males' allozyme type to determine whether or not the diploid male is sterile. Sterility of the diploid male is the subject for a future study.

It is still unknown whether the male-biased sex ratio of *D. semiclausum* under high temperature conditions is due to an increase in diploid males. This would obstruct the availability of *D. semiclausum* in the hot season when *P. xylostella* population increases. Further study is needed to determine whether emergence of diploid males is affected by rearing temperatures.

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