COMPLEMENTARY SEX DETERMINATION IN HYMENOPTERAN PARASITOIDS AND ITS IMPLICATIONS FOR BIOLOGICAL CONTROL

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Abstract   In haplodiploid Hymenoptera , unfertilized eggs produce haploid males while fertilized eggs lead to diploid females under most circumstances. Diploid males can also be produced from fertilization under a system of sex determination known as complementary sex determination (CSD). Under single2locus CSD , sex is determined by multiple alleles at a single sex locus. Individuals heterozygous at the sex locus are female while hemizygous and homozygous individuals develop as haploid and diploid males , respectively. In multiple2locus CSD , two or more loci , each with two or more alleles , determine sex. Diploid individuals are female if one or more sex loci are het2 erozygous , while a diploid is male only if homozygous at all sex loci. Diploid males are known to occur in 43 hym2 enopteran species and single2locus CSD has been demonstrated in 22 of these species. Diploid males are either developmentally inviable or sterile , so their production constitutes a genetic load. Because diploid male production is more likely under inbreeding , CSD is a form of inbreeding depression. It is crucial to preserve the diversity of sex alleles and reduce the loss of genetic variation in biological control. In the parasitoid species with single2locus CSD , certain precautionary procedures can prevent negative effects of single2locus CSD on biological control.

Key words   Complementary sex determination (CSD) , Hymenoptera , diploid males , biological control

1   INTRODUCTION

Biological control of insect pests and weeds frequently encounters the reduction of genetic diver2 sity during the process of collection , rearing , re2 lease and ultimate establishment of natural enemies. This reduction may interfere with the success of classical biological control programs (Mackauer 1976 , Unruh *et al*. 1983 , Roush 1990 , Hopper 1995 , Hopper *et al*. 1993 , Roush & Hopper 1995 , Morjan *et al*. 1999) and contribute partially to failure of some biological control programs.

Parasitic wasps (parasitoids) are widely used as biological control agents. The most common re2 production mode in parasitic hymenopterans is ar2

rhenotoky , in which males develop from unfertil2 ized , haploid eggs and females develop from fertil2 ized , diploid eggs. Haploid eggs receive a single set of maternal chromosomes ; diploid eggs contain two sets of chromosomes , one from each parent. Ovipositing females can determine the sex of their offspring by controlling the release of stored sperm from the spermatheca during reproduction. Howev2 er , some hymenopterans employ other reproductive modes : deuterotoky and thelytoky. Unfertilized eggs can produce either males or females in deuterotoky , while under thelytoky , unfertilized eggs give rise exclusively to females.

A number of parasitoid species show variation of sex ratios , especially under laboratory condi2

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tions. Thus , efficient economic usage will benefit from an understanding of the inherent sex determi2 nation mechanisms in parasitoids (Stouthamer *et al*. 1992 , Cook and Crozier 1995) . A clear under2 standing of these complex mechanisms will also shed light on the variation of sex ratios.

The proposed genetic mechanisms of sex deter2 mination in Hymenoptera are diverse and complicat2 ed (Beukeboom 1995) . Four general modes of sex determination have been proposed for the Hym2 enoptera : complementary sex determination (CSD) (Whiting 1939 , 1943 , Crozier 1971 , 1977) , genic balance (Cunha and Kerr 1957) , nucleo2cytoplas2 mic balance (Crozier 1971) , and genomic imprint2 ing sex determination (GISD ; Poire *et al*. 1992 , Beukeboom 1995 , Dobson and Tanouye 1998) . CSD has been confirmed in several widely distribut2 ed species in Hymenoptera. Under CSD , sex is de2 termined by multiple alleles located at one or more sex determining loci. Individuals that are heterozy2 gous on at least one sex locus develop as diploid fe2 males and hemizygous or homozygous individuals develop as haploid or diploid males , respectively (Whiting 1939 , 1943 , Stouthamer *et al*. 1992 , Cook 1993b) . The genic balance model hypothesi2 zes that male2determining genes do not have an ad2 ditive effect , unlike female2determining genes. Un2 der this scenario , diploidy would activate female2 determining genes , but not male2determining genes. The nucleo2cytoplasmic balance model as2 sumes that sex will be determined by a balance be2 tween nuclear and cytoplasmic products. The genomic imprinting model relies on a sex2determin2 ing locus that binds a product only when it is pr2 esent in male gametes. The gene turning on the fe2 male pathway is activated by the binding of this product to the sex locus , whereas non2binding leads to male development. Unfertilized haploid eggs are always male because they arise from female gametes which do not bind product , and fertilized diploid eggs are female because the bound product is intro2

duced with sperm. GISD was proposed as an alter2 native to CSD for those hymenopterans in which CSD was absent. Some species in Chalcidoidea , Chrysidoidea and Cynipoidea do not exhibit CSD and apparently have some other mechanisms of sex determination (Schmieder and Whiting 1947 , Whit2 ing 1960 , Skinner and Werren 1980 , Luck *et al*. 1992 , Cook 1993a , b) . Under the hypothesis of GISD (Poire *et al*. 1992 , Beukeboom 1995) , the paternal vs. maternal development involves different imprints by one or more sex loci. Sex is determined by the sex2specific imprinting of a sex2determining locus (S) binding a product (P) in the eggs or zy2 gotes. Unfertilized eggs containing only maternally derived autosomes with the" maternal imprint" de2 velop as haploid males. Fertilized eggs containing paternally imprinted autosomes and maternally de2 rived autosomes develop as females. However , only *Nasonia vitripennis* has been tested for GISD (Dob2 son and Tanouye 1998) . It is not clear to what ex2 tent GISD occurs in other taxa without CSD , and therefore , more tests in a wider scope are required. So far , there is good evidence for single2locus CSD in numerous systems (see table 1) , and evidence consistent with genomic printing for *Nasonia vitrip2 ennis* (Dobson and Tanouye 1998) . For the remain2 der of hymenopteran species , the mode of sex deter2 mination is unknown.

2   COMPLEMENTARY SEX DETERMINA2 TION (CSD)

Two patterns of CSD , single2locus CSD (*sl2* CSD) and multiple2locus CSD (*ml2*CSD) , have been proposed , of which *sl2*CSD has been con2 firmed in numerous species in various hymenopteran taxa. In *sl2*CSD , the sex ratio (proportion males) changes abruptly following one generation of in2 breeding when mother2son or father2daughter mating is conducted and then stabilizes , while in *ml2*CSD , the sex ratios increase gradually with subsequent generations of inbreeding.

Table 1 Diploid males tested in Hymenoptera to date (" + " or " - " indicates the species with or without diploid males , re2 spectively. Higher2level classification follows Goulet and Huber 1993 , Whitfield 1998).

Taxon	Occurrence of diploid males	Explanation	Reference
Sub2order Symphyta			
Tenthredinoidea			
Tenthredinoidae			
Athalia rosae	+	*sl*2CSD	Naito and Suzuki 1991
Neodiprion nigroscutum	+	*sl*2CSD	Smith and Wallace 1971
Neodiprion pinetum	+	*sl*2CSD ?	Stouthamer *et al*. 1992
Sub2order Apocrita			
Infra2order Parasitica			
Ichneumonoidea			
Braconidae			
Aphidius colemani	+ ?	*sl*2CSD	Ode *et al*. (unpublished data)
Cotesia rubecula	+	*sl*2CSD	Stouthamer *et al*. 1992
Habrobracon hebetor	+	*sl*2CSD	Inaba , 1939 , Whiting 1961
Habrobracon sp. near *hebetor*	+	*sl*2CSD	Holloway *et al*. 1999
Habrobracon serinopae	+	*sl*2CSD	Clark and Rubin 1961 , Clark *et al*. 1963
Heterospilus prosopidis	-		Wu *et al*. (unpublished data)
Microplitis croceipes	+	*sl*2CSD	Steiner and Teig 1989
Ichneumonidae			
Bathyplectus curculionis	+	*sl*2CSD ?	Unruh *et al*. 1984
Diadegma armillata	+	*sl*2CSD	Butcher *et al*. 2000b
Diagdema chrysostictos	+	*sl*2CSD	Butcher *et al*. 2000a
Diadegma eucerophaga	+	*sl*2CSD	Butcher *et al*. 2000b
Diadegma fabricianae	+	*sl*2CSD	Butcher *et al*. 2000b
Diadegma fenestralis	+	*sl*2CSD	Butcher *et al*. 2000b
Diadegma insulare	+	*sl*2CSD	Butcher *et al*. 2000b
Diadegma semiclausum	+	*sl*2CSD	Butcher *et al*. 2000b , Noda 2000
Diadromus pulchellus	+	*sl*2CSD	Hedderwick *et al*. 1985 , Periquet *et al*. 1993
Venturia canescens	+	*sl*2CSD	Beukeboom 2001
Chalcidoidea			
Eulophidae			
Melittobia chalybii	-		Schmieder 1938
Melittobia spp.	-		Schmieder and Whiting 1947 , Whiting 1947
Pteromalidae			
Dinarmus vagabundus	-		Rojas2Rousse *et al*. 1988
Muscidifurax raptor	-		Legner 1979 , Fabritius 1984
Myscidifurax zaraptor	-		Legner 1979

(continued)

Taxon	Occurrence of diploid males	Explanation	Reference
Nasonia vitripennis	+	Mutation	Whiting 1960, Skinner and Werren 1980, Dobson and Tanouye 1998
Pteromalus puparum	-		Whiting 1940
Cynipoidea			
Cynipidae			
Cothonaspis boulardi	-		Biemont and Bouletreau 1980
Diplolepis rosea	+	Gamete duplication	Stille and Davring 1980
Leptopilina heterotoma	-		Hey and Gargiulo 1985
Proctotrupoidea			
Scelionidae			
Telonomus fariai	-		Dreyfus and Breuer 1944, Cook 1993b
Infra2order Aculeata			
Apoidea			
Apidae			
Apis cerana	+	*sl*2CSD	Woyke 1979, Hoshiba *et al*. 1981
Apis mellifera	+	*sl*2CSD	Mackensen 1951, Woyke 1963, 1965
Augochlorella striata	+	*sl*2CSD	Parker and Owen 1990, Mueller 1993
Bombus atratus	+	*sl*2CSD	Garofalo 1973, Garofalo and Kerr 1975
Lasioglossum zephyrum	+	*sl*2CSD ?	Kukuk and May 1990
Melipona compressipes	+	*sl*2CSD ?	Kerr 1987
Melipona quadrifasciata	+	*sl*2CSD	de Camargo 1979
Melipona scutellaris	+	*sl*2CSD	Carvalho *et al*. 1995
Trigona quadrangular	+	*sl*2CSD ?	Cook 1993b
Chrysidoidea			
Bethylidae			
Goniozus nephantidis	-		Cook 1993a
Vespoidea			
Formicidae			
Doronomyrmex kutteri	+	*sl*2CSD ?	Cook 1993b
Epimyrma stumperi	+	*sl*2CSD ?	Cook 1993b
Formica aquilonia	+	*sl*2CSD ?	Cook 1993b
Formica pressilabris	+	*sl*2CSD ?	Pamilo and Rosengren 1984
Harpagoxenus sublaevis	+	*sl*2CSD ?	Cook 1993b
Lasius alienus	+	*sl*2CSD ?	Pearson, 1983
Leptothorax muscorum	+	*sl*2CSD ?	Loiselle *et al*. 1990
Pseudolasius sp. near *emeryi*	+	*sl*2CSD ?	Hung *et al*. 1972
Rhytidoponera chalybaea	+	*sl*2CSD ?	Ward 1980
Rhytidoponera confusa	+	*sl*2CSD ?	Ward 1980
Solenopsis invicta	+	*sl*2CSD ?	Hung *et al*. 1972, 1974
Vespidae			
Liostenogaster flavolineata	+	*sl*2CSD ?	Cook 1993b
Mischocytarrus immarginatus	+	*sl*2CSD ?	Queller *et al*. 1992

Note : The question marks indicate the data are not conclusive.

211　Single2locus CSD (sl2**CSD**)

Single2locus CSD was first proposed in the braconid wasp *Habrobracon* (= *B racon*) *hebetor* by Whiting (1939 , 1943) . In *sl2*CSD , sex is deter2 mined by multiple alleles at a single locus. Haploid (hemizygous) individuals are male with only one al2 lele at the sex locus. Diploid individuals can be ei2 ther homozygous or heterozygous at the sex locus. Individuals heterozygous at sex locus are females while homozygous individuals develop as diploid males (Cook and Crozier 1995) .

There are three mating modes under *sl2*CSD : unmatched mating , matched mating , and diploid male mating (Cook 1993b) . In unmatched mat2 ings , females and haploid males do not share a sex allele and all diploid offspring are females. In matched matings , a female mates with a haploid male that carries a sex allele identical with one of her own , and 50 % of diploid offspring are diploid males (Cook and Crozier 1995) . In species , *e*. *g*. *Athalia rosae* and *Neodiprion nigroscutum* , which produce viable diploid males , diploid males fre2 quently have difficulty in mating to females because of their bigger body size and incapability of insemi2 nating females (Smith and Wallace 1971 , Naito and Suzuki 1991) . However , matings between female and diploid males may occur as well (*Diadromus pulchellus* ; El Agoze and Periquet 1993 , El Agoze *et al*. 1994) . In this case , females have a lower fecundity compared to other females mated to hap2 loid males or to virgin females. Only haploid sons are produced due to the failure of insemination by diploid sperm and the functional virginity of mated females , or if diploid males are successful in insem2 inating diploid females , the resultant offspring will be haploid males (e. g. *D*. *pulchellus* ; El Agoze *et al*. 1994) or sterile triploids (e. g. *Athalia ro2 sae* , Naito and Suzuki 1991 ; Stouthamer *et al*. 1992) .

212　Multiple2locus CSD (ml2**CSD)**

Crozier (1971) proposed single2locus CSD as a special case of multiple2locus CSD , in which sex is determined by multiple loci each with multiple alleles. Diploids are female if one or more sex loci are heterozygous , whereas diploid individuals are male only if homozygous at all loci. Inbreeding leads to the production of diploid males but more gradually with continual generations of inbreeding under *ml2*CSD than under *sl2*CSD. Single2locus CSD can be tested by one inbreeding generation compared to outcross families , while more genera2 tions of inbreeding are required to test *ml2*CSD. The number of sex loci can be estimated by the gen2 erations of sib2mating required to cause diploid male production (Cook 1993a) . Nevertheless , *ml2*CSD has not been demonstrated in any species and there2 fore remains a hypothetical possibility.

3　HYMENOPTERAN SPECIES WITH DIP2 LOID MALES

Since the presence of diploid males was first discovered in *Habrobracon hebetor* by Whiting and Whiting (1925) , diploid males have been reported from about 43 species in six out of eight hym2 enopteran superfamilies tested , and *sl2*CSD was confirmed in at least 22 of these species (Stoutham2 er *et al*. 1992 , Cook 1993b , Periquet *et al*. 1993 , Cook and Crozier 1995 , Butcher *et al*. 2000a , b , Beukeboom 2001) (see table 1) . The diploid males in *Nasonia vitripennis* (Chalcidoidea) and *Diplolepis rosea* (Cynipoidea) were caused by mutation and gamete duplication respectively , not from inbreeding , and thus , cannot be attributed to CSD (Table 1) . The *sl2*CSD and *ml2*CSD models have been explicitly tested only in *Nasonia vitripen2 nis* (Skinner and Werren 1980) , *Goniozus nephan2 tidis* (Cook 1993a) , *Diadromus pulchellus* (Pe2 riquet *et al*. 1993) , and *Aphidius colemani* (Ode *et al*. unpublished data) , but these four species were shown not to conform to *ml2*CSD. However , *ml2*CSD cannot be excluded from the Hymenoptera based on current evidence , because some biases may exist against detection of *ml2*CSD , such as le2 thality of males homozygous at some of these loci (Cook 1993b) . Another question is whether *ml2* CSD can be excluded in species for which *sl2*CSD exists. Only *Habrobracon hebetor* (= *juglandis*) (Whiting 1943) , *Diadromus pulchellus* (Periquet

et al. 1993) and *Solenopsis invicta* (Ross and Fletcher 1985) have thus far been specifically ex2 amined , and *ml2*CSD was refuted in these three species.

Beukeboom *et al*. (2000) excluded *sl2*CSD from two braconid species , *Asobara tabida* and *Al2 ysia manducator* based on the comparison of brood size and sex ratio between inbred and outbred fami2 lies. But it is unknown whether there is *ml2*CSD in these two species without further testing even though their evidence shows no *sl2*CSD in these two spe2 cies. It would be particularly interesting if further examination on *ml2*CSD is conducted , since *sl2*CSD is hypothesized to be ubiquitous in the superfamily Ichneumonoidea (containing families Braconidae and Ichneumonidae) , a sister group of Aculeata , and these two braconid species are the first cases in this superfamily where *sl2*CSD is absent. Our strict2 ly designed inbreeding test of CSD in another bra2 conid species , *Heterospilus prosopidis* , showed that both *sl2* and *ml2*CSDs are absent from this species (Wu *et al*. unpublished data). No diploid males were observed , and the comparison of offspring size , sex ratio or proportion of all2male families be2 tween inbreeding and outbred lines over 13 consec2 utive generations did not conform to expectations under CSD.

Male2biased sex ratios were apparently not found in response to prolonged inbreeding in several species that presumably sib2mate in nature (Stouthamer *et al*. 1992) , and CSD can be ruled out in these taxa (e. g. , superfamilies Chal2 cidoidea , Chrysidoidea , Cynipoidea and Proc2 totrupoidea) (Table 1). Based on theoretical and experimental evidence , *sl2*CSD seems unlikely to be present in those highly inbreeding species with female2biased sex ratios (Stouthamer *et al*. 1992 , Cook 1993c) since CSD will lead to the production of diploid males and male2biased sex ratio under in2 breeding systems.

4   DETECTION OF CSD AND DIPLOID MALES

Two methods can be used to identify CSD.

First , inbreeding effects on sex ratio and develop2 mental mortality can be compared between inbred and outbred families. In inbred lines , mother2son matings can be set up by allowing virgin females to oviposit and mate to their haploid sons when they reach adulthood. If it is difficult to keep mothers alive long enough to mate with their sons , brother2 sister crosses can be used instead. In this case , more inbreeding generations may be required to test CSD compared with the test initiated by mother2son mating because only one half of the matings are ex2 pected to be matched. Sex ratios (proportion males) produced by inbred females are expected to be higher than that produced by outbred females if CSD is operating. If started with a mother and son mating , 10 consecutive generations of sib2mating will be enough to detect a system of involving at least 15 independent loci (Cook 1993a).

In *sl2*CSD , the use of controlled matings be2 tween relatives permits easy and rapid quantitative predictions. For example , broods with no diploid males and broods with equal numbers of females and diploid males should be equally common following sib2matings if their parents did not share a sex al2 lele. The diploid offspring of any mother and son mating should be 50 % male. Furthermore , diploid males will be expected from any mating between fe2 males and their haploid male brothers from a brood that contains diploid males (Cook 1993b).

Inbreeding should increase diploid male pro2 duction because it increases the incidence of matched mating and homozygosity. Diploid males produce diploid sperm and are effectively sterile , imposing a genetic load on the population (Cook and Crozier 1995). These diploid males are either inviable resulting in offspring mortality , or sterile if they reach adulthood. The survival of diploid males is rather common (Woyke 1962 , 1969 , Petters and Mettus 1980 , de Camargo 1982 , Kukuk and May 1990 , El Agoze and Periquet 1993 , Duchateau *et al*. 1994 , Holloway *et al*. 1999 , Beukeboom 2001) and diploid males are often capable of mating as well (El Agoze *et al*. 1994 , Holloway *et al*. 1999). For instance , diploid males of *Diadromus*

pulchellus have normal viability, produce diploid sperm and are able to inseminate, but the resulting offspring are haploid sons due to the sterility of dip2 loid males (El Agoze and Periquet 1993, El Agoze *et al*. 1994).

Second, the presence of CSD will be support2 ed by the finding that diploid males are produced. Diploid males can be identified using the following methods: (1) Paternal inheritance of genetic mark2 ers, including visible genetic markers and dominant and co2dominant molecular markers. Visible reces2 sive markers were used frequently to identify diploid males (Inaba 1939, Risman 1942, Whiting 1943, Grosch 1945, Whiting 1961, Holloway *et al*. 1999). All haploid male offspring have the reces2 sive marker of their mother, whereas the diploid male offspring also have the dominant phenotype of their father. Diploid males can be recognized in crosses where females homozygous for a recessive marker are mated with males of dominant wild type. Diploid males can also be detected by the presence of two variants of one enzyme system using electro2 phoresis (Hung *et al*. 1972, Unruh *et al*. 1984, Hedderwick *et al*. 1985, Steiner and Teig 1989, Hunt and Page 1994, Noda 2000, Butcher *et al*. 2000a, Beukeboom 2001). Polymorphic RAPD2 PCR loci can also be used to assign paternity in male offspring (Hunt and Page 1994, Holloway *et al*. 1999, 2000). (2) Cytology, karyotyping of pupal or adult testes tissues can be used to directly identify diploid males (Naito and Suzuki 1991). (3) DNA quantitation. Butcher *et al*. (2000 a, b) used flow cytometry technique to compare the DNA content of single2cell brain neuron between haploids and diploids. (4) Male size dimorphism (Inaba 1939, Grosch 1945, Smith and Wallace 1971, Chaud2Netto 1975, Ross and Fletcher 1985, Naito and Suzuki 1991, Periquet et al. 1993). In some species, diploid males have larger cells and are bigger than haploid males. For example, diploid males of *Habrobracon hebetor* can be recognized by their larger cell size, as measured by hair density on the wings (Whiting 1943). Cell size of diploid male is larger than that of haploid male or diploid

female and this has been suggested as an explana2 tion for the differential mortality of diploid males (Grosch 1945).

However, the above four methods cannot be applied to all hymenopteran species with diploid males, because diploid males may die during devel2 opment or adult body size and coloration may be greatly dependent on the host species and rearing temperature (Fisher 1959, Butcher *et al*. 2000b). Butcher *et al*. (2000b) found that even wing hair density is also unreliable due to the overlap in the size distribution. Furthermore, Butcher *et al*. (2000a, b) were skeptical about the reliability of morphological characteristics for haploid2diploid discrimination, and suggested using flow cytometric neuronal cell DNA analysis to identify diploid males, since this DNA analysis can easily discrimi2 nate haploid and diploid individuals, and neuronal cell DNA content is not related to temperature or host2status.

5   CSD AND EVOLUTION OF SEX DETER2 MINATION MECHANISMS

The wide distribution of CSD in the suborders Symphyta and Apocrita and in the infra2orders Ac2 uleata and Parasitica suggests that this form of sex determination may be ancestral in Hymenoptera (Cook 1993a, b). Butcher *et al*. (2000b) also supported the hypothesis that *sl*2CSD is the ances2 tral sex determination mechanism in Hymenoptera (AculeataPIchneumonoidea), in which Ichneu2 monoidea is recently considered by molecular evi2 dences as a sister group of Aculeata (Whitfield 1998). However, our finding of absence of CSD in *Heterospilus prosopidis* raises an open question to this hypothesis, due to the relatively ancestralPbasal phylogenetic status of the subfamily Doryctinae (in which *H*. *prosopidis* is located) in the family Bra2 conidae (van Achterberg 1984). A plausible expla2 nation of the absence of CSD in this primitive spe2 cies might be that other unknown sex determining mechanism evolves and substitutes CSD.

Bull (1981) suggested that *sl*2CSD might be ancestral to *ml*2CSD. Periquet *et al*. (1993) noted

that multi2locus CSD might evolve from *sl*2CSD by tandem duplication of the sex locus closely linked on a chromosome , followed by dispersion of the re2 peated genes to other chromosomes. Other sex de2 termining systems might be derived from CSD over time with the eventual loss of CSD. For naturally inbreeding species , sex determination mechanisms other than CSD will be expected to avoid the pro2 duction of diploid males. A more complete under2 standing of the sex determination systems present in the Hymenoptera requires further tests in other su2 perfamilies.

Relative to *sl*2CSD , all of the proposed sex determination modes decrease or eliminate the pro2 duction of diploid males and may therefore especial2 ly advantageous at low population densities , where sex alleles diversity may be low. Selection for sex determination mechanisms other than *sl*2CSD may be stronger in parasitoid species that typically or periodically persist at low population densities. Therefore , the quest to find species exhibiting *ml*2 CSD or any modes of sex determination other than *sl*2CSD might be focused by looking at the parasi2 toid complexes of cyclic defoliators such as the gyp2 sy moth , populations of which can remain at very low levels for many years , then reach outbreak lev2 els in a relatively short time (Elkinton and Liebhold 1990) .

6   CSD AND BIOLOGICAL CONTROL

Diploid male production poses a potential threat to the success of biological control attempts using parasitoids. The occurrence of diploid males in a population reduces the potential growth rate of populations because some fertilized eggs become diploid male eggs that either die during development or become sterile males when they reach adulthood. In matched matings under *sl*2CSD , 50 % of fertil2 ized eggs will develop as diploid males or die during development. CSD therefore increases the apparent sex ratio (proportion males). The production of diploid males is equivalent to increased mortality of females , and therefore is unfavorable for biological control. High ratio of males (i. e. , less female in2

dividuals) in a population has a strong , negative in2 fluence on the population growth , because only fe2 males produce offspring and effectively contribute to the suppression of insect pests.

Allelic diversity at the sex locus and mating patterns of parasitic wasps will determine heterozy2 gosity at the sex locus (Ross *et al*. 1993) and the population growth rate (Stouthamer *et al*. 1992) . Large populations likely harbor more sex alleles and consequently decrease the probability of diploid male production. Estimates of the number of sex al2 leles in natural populations lie between 3 and 86 (Table 2) . Limited initial field collections , genetic drift , inbreeding , population crashes in mass rear2 ing or other population bottlenecks may facilitate loss of sex alleles and reduction of genetic diversity (Unruh *et al*. 1984) , and might even result in a population with only two sex alleles at a single locus (Cook 1993a) . Petters and Mettus (1980) suspect2 ed that many laboratory populations of *Habrobracon hebetor* might be in this state. Predictions based on *sl*2CSD , i. e. , an increasing frequency of all2male families in the offspring of laboratory populations and a low establishment rate of populations released as biological control agents , occurs in the families Braconidae and Ichneumonidae but not in some oth2 er parasitoid taxa (Stouthamer *et al*. 1992 , Hopper 1995) , e. g. , the chalcidoids.

In response to these potential problems present in the practice of biological control , Stouthamer *et al*. (1992) proposed two solutions to overcome the loss of sex alleles during the laboratory augmenta2 tion : maintaining the parasitic wasps as a large pop2 ulation or keeping the wasps as a large number of isolated subpopulations (inbred lines) . The first strategy reduces the rate at which sex alleles are lost , and the second strategy results in many alleles being lost in each subpopulation , but at least two sex alleles are retained per subpopulation that exists (e. g. Whiting 1943 , Petters and Mettus 1980 , Cook 1993c) . However , Stouthamer *et al*. (1992) also pointed out there is a potential threat by CSD due to the population extinction caused by the pro2 duction of diploid males and their matings with fe2

Table 2   Estimated number of sex alleles in Hymenoptera.

Species	Estimated sex alleles number	References
Apis mellifera	19	Adams *et al*. 1977
Bombus terrestris	24 - 46	Dutchateau *et al*. 1994
Habrobracon hebetor	9 - 20	Whiting 1943 , Heimpel *et al*. 1999
Diadegma armillata	17 - 19	Butcher *et al*. 2000b
Diadegma chrysosctictos	17 - 19	Butcher *et al*. 2000a
Diadegma eucerophaga	7 - 10	Butcher *et al*. 2000b
Diadegma fabricianae	14 - 15	Butcher *et al*. 2000b
Diadegma fenestralis	4 - 6	Butcher *et al*. 2000b
Diadegma insulare	4 - 6	Butcher *et al*. 2000b
Diadegma semiclausum	4 - 6	Butcher *et al*. 2000b
Diadromus pulchellus	15	Periquet *et al*. 1993
Lasioglossum zephyrum	10 - 25	Kukuk and May 1990
Melipona compressipes	20	Kerr 1987
Melipona scutellaris	7 - 36	Carvalho 2001
Neodiprion nigroscutum	3	Smith and Wallace 1971
Solenopsis invicta	15 - 86	Ross and Fletcher 1985 , Ross *et al*. 1993

males , if the second strategy is employed. Cook (1993c) argued that inbred lines under CSD can be maintained without population crash provided that the populations are reared in large enough colonies to avoid extinction , and stated that inbred lines may be used as an additional resources of sex alleles to a single large population , not just as an alternative suggested by Stouthamer *et al*. (1992) . Mainte2 nance of genetic diversity by maintenance of multi2 ple isofemale lines has been confirmed for insects in general by Roush and Hopper (1995) .

Combining samples from different geographic regions may increase the sex alleles , provided that wasps from different geographical origins do not show crossing incompatibility. The success of estab2 lished populations with few sex alleles can be en2 hanced by importing and releasing additional wasps from other populations (Stouthamer *et al*. 1992) . Cook and Crozier (1995) stated that taking a good initial sampling of natural allelic diversity and mini2 mizing loss of genetic variation in the rearing and release will be more practical for a biological control

program. Keeping large populations , maintaining multiple inbred lines , reducing between2family variance in offspring production and avoiding popu2 lation crashes are very important practices.

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