Genetics of Sex Determination and the Improvement of Biological Control Using Parasitoids

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ABSTRACT Diploid males are known to occur in several braconid and ichneumonid species. These diploid males are the result of a single-locus, sex-determination mechanism. Heterozygotes at this sex locus develop into females, whereas homozygotes (haploids) and homozygotes (diploids) develop into males. Diploid males have a low fertility and their frequency drastically increases with small populations or inbreeding. The implications of this sex-determining mechanism for the use of parasitoids in biological control are explored. Production of diploid males leads to male-biased sex ratios and can reduce rates of establishment and population growth. Taxa in which a single-locus sex determination has been found (e.g., Ichneumonidae and Braconidae) often experience extreme male-biased sex ratios in mass rearing and have been more difficult to establish than taxa with other modes of sex determination (e.g., Chalcidoids). The effect of laboratory rearing on the number of sex alleles, frequency of diploid males, and population growth rates is explored by computer simulation. Methods of rearing and release that can enhance the number of sex alleles and the establishment of parasitoids are discussed. Furthermore, additional small-scale releases may enhance the effectiveness of already established populations by increasing number of sex alleles and the rate at which their population grows.

KEY WORDS Insecta, diploid male, biological control, rearing

NATURAL ENEMIES USED for biological control often go through genetic bottlenecks during collection, rearing, and subsequent establishment in the field. Such bottlenecks reduce genetic variability, which is thought to impede a population's ability to adapt to new environments and, therefore, its potential for the biological control (Unruh et al. 1983). In Hymenoptera, reduced genetic variability causes an additional problem: the production of diploid males from fertilized eggs. The occurrence of diploid males is a consequence of the sex-determining mechanisms in certain Hymenoptera-i.e., a single-locus, sex-determination mechanism first described by A. R. and P. W. Whiting and their students (reviewed in Whiting 1961). The Whitings studied sex determination in *Habrobracon hebetor* Say and found that haploid individuals were always males, whereas diploid individuals were generally females. However, under inbreeding conditions some diploid individuals were males. Diploid males, once known only from *H. hebetor* and some bees, have recently been reported in five species of Ichneumonoidea (Clark et al. 1963, Unruh et al. 1984, Hedderwick et al. 1985, Steiner & Teig 1989; W.W.M. Steiner, personal communication). The generality of this single-locus sex determination to the Ichneumonoidea still remains to be determined. Additionally, diploid males also have been found in three species of sawflies and several social hymenoptera. Chalcidoidea apparently have some other mechanisms of sex determination because diploid males do not appear when these species are inbred (Schmieder & Whiting 1946, Whiting 1960, Skinner & Werren 1980, Luck et al. 1992).

The purpose of this article is to call attention to the implications of the single-locus sex determination for biological control. We hypothesize that the relatively high rate of biocontrol failures with Ichneumonidae and Braconidae stems from the reduced genetic variability at the sex locus, caused in part by inbreeding during culturing and release, with the consequent appearance of diploid males. We present evidence for this hypothesis and propose solutions for the problems that this sex-determination mechanism causes in biological control.

Single-Locus Sex Determination

In single-locus sex determination, the sex of an individual is determined by alleles at a single

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sex locus. Several different alleles are found in a population. Haploid individuals have only one allele at the sex locus and are always males. Diploid individuals have two alleles at the sex locus and can be either homozygous (i.e., having two identical copies of an allele, for instance AA or BB) or heterozygous (i.e., having copies of two different alleles, for instance AB or AC). Homozygous individuals are males and heterozygous individuals are females. Under inbreeding conditions, when for instance a female (AB) mates with her son (either A or B), 50% of the fertilized eggs should give rise to diploid males (i.e., those that are either AA or BB). However, in experiments where *H. hebetor* females were mated with their sons, substantially <50% of the diploid offspring is male (Torvik 1931). Diploid males appear to suffer higher mortality than females (Petters & Mettus 1980), and at most, 31% of the diploid adult offspring are male in this species (Bostian 1934). The temperature at which offspring are reared probably affects the mortality of diploid males. Diploid males are less frequent in progeny reared at 20°C, compared with those reared at 30°C (Whiting & Anderson 1932).

Snell (1935) proposed an alternative explanation for the <50% males in diploid offspring of close crossed *H. hebetor.* He proposed that sex determination was not simply the result of one locus with several alleles, but that several loci, each with numerous alleles, were involved. Only individuals homozygous for all sex loci become diploid males. However, Horn (1943) experimentally excluded the multiple locus hypothesis in *H. hebetor.* Smith & Vikki (1978) reported that two loci were responsible for sex determination in the sawfly *Neodiprion nigriscutum* Middleton. Unfortunately, the data for this conclusion have not been published nor could they be retrieved from the late S. G. Smith's notes (D. R. Wallace, personal communication).

In North American *H. hebetor,* few diploid males survive to become adults and of these =90% are sterile (Whiting 1925, Torvik 1931). This sterility is caused by an inability of most diploid sperm to penetrate the eggs (MacBride 1946). Occasionally some do and a sperm successfully fertilizes an egg, but the resulting triploid females are sterile (Torvik 1931). In Japanese *H. hebetor (=pectinophorae)* (Inaba 1939), a much smaller percentage (20-40%) of diploid males is sterile. *Habrobracon serinopae* (Cherian) diploid males do not have a higher mortality, but their fertility is unknown (Petters & Mettus 1980). In the ichneumonid *Diadromus pulchellus* Wesmael, diploid sperm are able to penetrate the egg, but such fertilized eggs suffer high mortality rates (Chauvin et al. 1988). Diploid males in the sawfly *N. nigriscutum* do not suffer a higher mortality than diploid females; however, these males are incapable of successfully completing copulations (Smith & Wallace 1971). The diploid males of the sawfly *Athalia rosae L.* have a lower viability than diploid females, and they have a reduced fertility compared to haploid males (Naito & Suzuki 1991).

Evidence for single-locus sex determination can be gathered by several methods: distribution of sex ratios in sib matings, genetic identification of diploid males, or morphological identification of diploid males.

If single-locus sex determination applies to a species, a bimodal distribution of offspring sex ratios is expected in sib matings. In these sib matings, the male and the female either share one sex allele (homoallelic) or do not have a sex allele in common (heteroallelic). In the homoallelic crosses, a male-biased offspring sex ratio is expected because 50% of the diploid eggs become males. In the heteroallelic crosses, the offspring sex ratio should be substantially higher. This detection method only works if the variance in sex ratio produced within each group of crosses is low.

Diploid males can be distinguished genetically using either visual or electrophoresic markers. Visual markers were used by the Whitings and their colleagues (Whiting 1961). Diploid males can be recognized in crosses where females homozygous for a recessive marker are mated with males of dominant wild-type. All the haploid male offspring have the recessive marker of their mother, whereas the diploid male offspring have the dominant phenotype of their father. With the widespread application of allozyme electrophoresis, diploid males can be detected by the presence of two variants of one enzyme system (Hung et al. 1972, Unruh et al. 1984, Hedderwick et al. 1985, Steiner & Teig 1989).

Morphologically diploid males have larger cells and are larger than their haploid counterparts. *H. hebetor* diploid males can be recognized by their larger cell size, as measured, for example, by bristle density on the wings. Grosch (1945) shows that haploid and diploid males have nonoverlapping bristle density distributions. The pupae of diploid males in sawflies are heavier than those of haploid males (Smith & Wallace 1971, Naito & Suzuki 1991).

Distribution of Male Diploidy in Hymenoptera

No systematic search has been conducted for the applicability of the single-locus, sex-determination model to different species of Hymenoptera. The reported cases generally were detected by accident. The distribution of known cases of male diploidy is shown in Table 1. In the parasitic Hymenoptera, diploid males, in response to inbreeding, seem to be restricted to some of the Ichneumonoidea. Those reported in *Nasonia vitripennis* (Walker) are caused by a

Table 1. Listing of diploid males reported in Hymenoptera

Insect	Reference
Tenthredinoidea	
Athalia rosea	Naito & Suzuki 1991
Neodiprion nigroscutum	Smith & Wallace 1971
N. pinetunt (Norton)	Wallace, personal communication
Ichneumonoidea	
Braconidae	
Habrobracon hebetor	Whiting 1961, Inaba 1939
H. serinopae	Clark et al. 1963
Cotesia rubecula Marshall	Steiner, personal communication
Micropletis croceipes (Cresson)	Steiner & Teig 1989
Icheumonidae	
Bathyplectes curculionis (Thomson)	Unruh et al. 1984
Diadromus pulchellus	Hedderwick et al. 1985
Chalcidoidea	
Nasonia vitripennis	Whiting 1960
Apoidea	
Apis mellifera	Mackensen 1951
Apis cerana	Woyke 1979
Bombus atratus	Garbfalo 1973
Melipona quadrifasciata	de Camargo 1979
Augochorella striata	Packer & Owen 1990
Lasioglossum zephyrum	Kukuk 1989
Formicidae	
Pseudolasius sp.	Hung et al. 1972
Rhytidoponera spp.	Ward 1980
Solenopsis spp.	Hung et al. 1974
Lasius alienuslniger	Pearson 1983
Formica pressilabris	Pamilo & Rosengren 1984

Table 2. Cases where inbreeding did not lead to male-biased sex ratios as predicted by single-locus sex determination

Insect	Reference
Chalcidoidea	
Mellitobia sp.	Schmieder & Whiting 1947, Whiting 1947
Nasonia vitripennis	Skinner & Werren 1980
Muscidifurax raptor (Girault & Sanders)	Fabritius 1984
Dinarmus vagabundus	Rojas-Rousse et al. 1988
Cothonaspis boulardi	Biemont & Bouletreau 1980
Leptopilina heterotoma	Hey & Cargiulo 1985

mutation and, therefore, are not an example of single-locus sex determination (Whiting *1960).* Most parasitic Hymenoptera, other than Ichneumonoidea, probably have a sex-determining mechanism that differs from single-locus sex determination (Luck et al. *1992).* The few cases found in the Ichneumonidae and Braconidae suggest that single-locus sex determination characterizes these families; however, we clearly need more data on its prevalence within these taxa. On theoretical and empirical grounds it seems likely that single-locus sex determination is restricted to those species that generally practice outbreeding. Male-biased sex ratios were not found in response to prolonged inbreeding in several species that normally sib mate in nature (see Table 2).

Population Effects of Single-Locus Sex Determination

The occurrence of diploid males in a population reduces the potential growth rate of the populations because some fertilized eggs become diploid male eggs that either die during development or become male adults. These diploid males would normally have been females. Also, females inseminated by diploid males produce only haploid sons or triploid (sterile) daughters. How severely the population growth rate is affected depends on two factors: (1) the number of alleles at the sex locus and (2) the mating system.

In *H. hebetor, 9* different alleles have been found (Whiting *1943, 1961)* in the honeybee, *Apis mellifera L., 8-19* (Adams et al. *1977)* and in the fire ant, *Solenopsis invicta* Buren, 15 (Ross & Fletcher *1985).* In *H. hebetor,* the number of alleles was determined by crossing different lines, while in the honeybee and the fire ant the number was statistically derived from the incidence of diploid males.

The proportion of diploid sons produced depends on the relatedness of individuals participating in the crosses. If mating is random in a population with *n* sex-alleles, selection will result in an equilibrium frequency of *1/n* for each sex allele (Yokoyama & Nei *1979).* In such a population, the chance that a female mates with a male that carries one of her sex alleles *is 2/n.* Therefore, the fraction of males among the diploid offspring can be substantial. In situations where strict sib mating occurs, this fraction increases dramatically, and the percentage of males among the diploids should approach *1/2.*

To illustrate the effects of a reduced number of alleles on important population characteristics such as sex ratio and rate of increase, formulas were derived for a large, randomly mating population containing *n* alleles. The sex ratio *(SR)* (percentage female offspring) is a function of the fertilization rate of eggs (F)-i.e., the percentage of eggs that are fertilized-and the number of alleles. The rate of increase of the population is expressed as the number of daughters produced per female (R), which is a function of the number of eggs laid (E), the fertilization rate of the eggs (F), and the survivorship of the offspring.

When diploid males survive but do not participate in mating, as in *N. nigroscutum* (Smith & Wallace *1971),* the relationships at equilibrium are:

$$SR(n) = F*(1 - 1/n) \quad (1)$$

$$R(n) = SR(n)*E = F*(1 - 1/n)*E \quad (2)$$

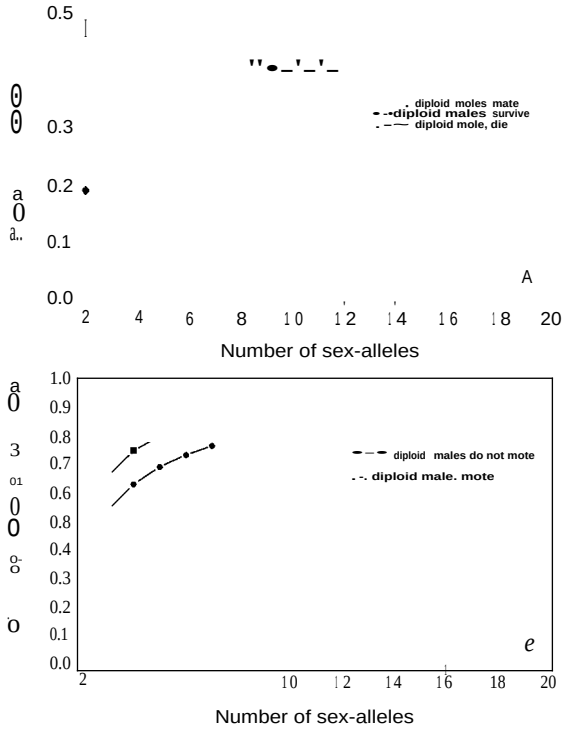

Fig. 1. (A) Relationship between number of sex alleles and the offspring sex ratio in a large, randomly mating population with an egg fertilization rate of 50%, when diploid males mate but their sperm fails to fertilize eggs (•); diploid males survive but do not mate (•); and diploid males die before reaching adulthood (A). See formulas 1, 3, and 5. (B) Relationship between the relative rate of population growth in a randomly mating population with an egg fertilization rate of 50%, when diploid males mate (•) or do not mate (•). Growth rate equals 1 when all fertilized eggs become females. See formulas 2, 4, and 6.

When all the diploid males die before reaching adulthood, as occurs in H. *hebetor* (Petters & Mettus 1980), the relationships at equilibrium are:

$$SR(n) = (n - 1)*F/(n - F) \quad (3)$$

$$R(n) = F*(1 - 1/n)*E \quad (4)$$

When the diploid males survive, are sterile, participate in mating, and the females they mate with produce all male (haploid) offspring the relationships at equilibrium become:

$$SR(n) = 1 - [n^2(1 - F) + \sqrt{(n^4(1 - F)^2 + 4n^3F)}]/2n^2 \quad (5)$$

$$R(n) = SR(n)*E \quad (6)$$

Figure 1 shows these relationships for the case where 50% of the eggs are fertilized. In all cases, the sex ratio (% females) and the population growth rate are depressed. This depression can be substantial when few sex alleles occur in the population.

Single-Locus Sex Determination and Biological Control

When a population is imported for biological control and is cultured in the laboratory for several generations, sex alleles are likely to be lost from the culture. Initially, the field sample probably contains only a subset of the sex alleles present in the population from which the imported individuals were collected. A further reduction occurs through genetic drift and inbreeding during the laboratory rearing, particularly if the cultures experience periodic reductions in population size (Unruh et al. 1984). The loss of sex alleles results in two detrimental effects on these biological control agents: (1) a male-biased sex ratio and (2) a reduced growth rate of the wasp population.

The frequency of females in a parasitoid population is important because only females are effective biological control agents. The number of female offspring produced per female is also an important determinant of a population's growth rate; the more daughters produced per mother, the faster a population can increase and the faster a pest is suppressed. Also, the higher the population's growth rate the better the chance that the wasps become established in the first place. Small differences in the growth rate of the released agent make a large difference in the abundance of the parasitoid in a few generations. For instance the production of seven versus five daughters per female results in a more than a 5-fold difference in total number of females in the fifth generation.

We suspect that populations with a low number of sex alleles, manifest low sex ratios (percentage females) during rearing and are more difficult to establish in the field. Cursory inspection of the literature concerning laboratory cultures of Ichneumonidae and Braconidae indeed show that they often produce low sex ratios («50% females) (Simmonds 1947, Platner & Oatman 1972, Rappaport & Page 1985, Smith et al. 1990, Grinsberg & Wallner 1991). These low sex ratios are attributed to mating problems during mass rearing, which causes a high frequency of virgins among the females. Sex determination genetics has not been considered as a possible cause of male-biased sex ratios in any of these cases.

To test whether an association exists between families in which single-locus sex determination has been found and the success of establishment, we compared the establishment rate of Ichneumonoidea versus Chalcidoidea from a world review of biological control (Clausen 1978). In this analysis, we chose the biological control at-

Table 3. Rate of establishment after introduction of Braconidae, Ichneumonidae, and Chalcidoidea for the biological control of Lepidoptera (based on Clausen et al. [1978] [see text])

Insect	Establishment	Failure
Ichneumonoidea	82	231
Braconidae	61	147
Ichneumonidae	21	84
Chalcidoidea	40	29

tempts on Lepidoptera because of the large number of Braconidae and Ichneumonidae that were used. Only those cases for which the status of establishment was definitely determined were included. A datum point consisted of a parasitoid species per country. If a species was released more than once in a country, with several failures and only one establishment, the parasitoid for that country was scored as a positive establishment. Classification of the parasitoid species to superfamily or family is according to Krombein et al. (1979).

Significantly fewer Ichneumonidea than Chalcidoidea became established ($\chi = 26.2$, df = 1, $P < 0.005$) (Table 3). The detrimental effects of diploid males on the population growth rate are consistent with this finding, but other factors may also contribute to the lower establishment rate of Ichneumonoidea. These include longer generation time of Ichneumonoids versus Chalcidoids. Ichneumonoids may parasitize the less-abundant later instars of hosts, whereas Chalcidoids may oviposit in the more-abundant earlier stages.

Simulations of the Effects of Importation and Rearing on the Loss of Sex Alleles

The first step of a biological control project consists of collecting wasps from a field population and then rearing them in a laboratory culture, to satisfy quarantine regulations and often to increase the parasitoid's population size. Alleles can be lost during the collection of the wasps from the field population, the culturing in the laboratory, and the release of the mass-reared wasps in the field. To investigate the effects of traditional importation and rearing practices on the number of sex alleles in a population, several computer simulations were executed. The assumptions of these simulations are detailed later.

In the first simulation we determine how the size of the initial collection affects the number of sex alleles in the sample. We do not know the number of sex alleles in natural populations; however, this number has been estimated to be between 15 and 20 in two imported populations (Adams et al. 1977, Ross & Fletcher 1985). Both populations probably have a reduced number of alleles because of recent population bottlenecks. Theoretical studies indicate that populations

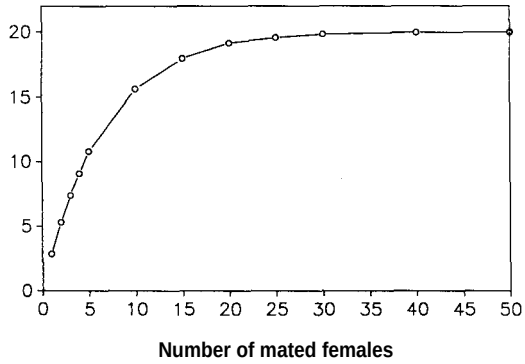

Fig. 2. Relationship between the number of alleles found in a sample of n mated females taken from a randomly mating population with 20 sex alleles.

with effective sizes of ~5,000 should be able to maintain >30 different sex alleles (Yokoyama & Nei 1979). For our simulations we assumed that the population contained 20 different alleles and each was maintained at a frequency of 1/20. Random mating was assumed and we randomly chose n mated females and determined the number of alleles present in a sample (Fig. 2). Fig. 2 shows that a sample of 20-30 mated females contains almost all of the alleles present in the population. These samples represent an idealized situation; when wasps are collected from the field they are often not collected as mated females but as parasitized hosts. In such cases the same number of alleles can be collected by mating each virgin female with a different male. In gregarious species, all the offspring from a parasitized host most likely represent the offspring of a single mated female.

After collection and initiation of a laboratory culture for propagation, further losses of sex alleles may occur over time. In simulations, we studied a number of factors facilitating such losses: small population size, most of the offspring produced by a few females, and most matings arise from a few males.

To simulate the effect of population size, a sample of mated females from the field is taken to the laboratory where it is maintained at population sizes equal to the field sample. Each generation, pairs are randomly chosen from the offspring of the previous generation, and each mated female is allowed to produce the same number of offspring. If diploid males are formed they are excluded from mating. These simulations (Fig. 3) indicate that the larger the laboratory population the slower the loss of alleles, but that the fewer generations the culture is maintained in the laboratory the fewer alleles are lost. Generally, not all females contribute the same number of offspring to the next generation. If variance is added to the number of offspring produced per female, the loss of alleles occurs more rapidly (Fig. 4). In laboratory populations, some

Fig. 3. Relationship between the mean number of sex alleles present in the population and the number of generations the population was maintained at 10, 25, 50, or 100 pairs. Error bars represent the standard deviation around the mean. Mean and standard deviation are based on 100 replicates.

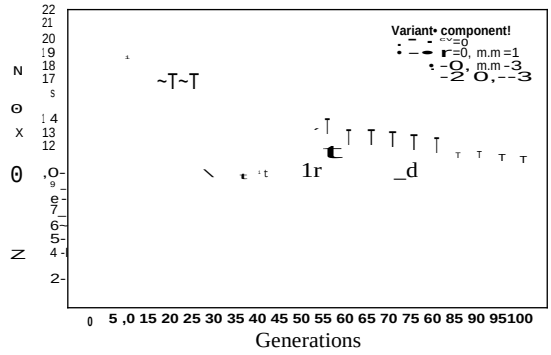

Fig. 5. Relationship between the mean number of sex alleles present in the population and the number of generations this population was maintained at 100 mated females, when the number of offspring produced by each pair is constant, or varies with a CV of 100 or 200 and the number of matings each male participates in varies like a Poisson variable (mean number of coatings [m.m.] = 1 or 3). Error bars represent the standard deviation around the mean. Mean and standard deviation are based on 100 replicates.

males may mate with more than one female, whereas other males do not mate at all. We next added the variance in male matings. This also increases the loss of alleles in that population (Fig. 5).

Other factors (not simulated) also contribute to the loss of alleles in such laboratory populations. These include participation of diploid males in the matings, variance in offspring sex ratio, and nonrandom coatings-i.e., preferential matings between siblings. Clearly the simulations show the potential loss of alleles that can occur during laboratory rearings. The effect of this reduced number of sex alleles on the rate of population growth can be calculated with equations 1-6.

Assumptions Used In Simulations

In the simulations to determine the mean number of alleles per sample (Fig. 1), the following assumptions were made. All alleles occurred at a frequency of 1/20 (20 is the number of alleles). In the population from which the randomly mated females were drawn, only heterozygous diploid individuals existed. The genotype of a mated female was determined by randomly sampling the sex-allele distribution in which each allele had the same chance of being selected. If two identical alleles were selected, that individual was discarded, and a new individual was randomly selected until a heterozygous individual was sampled. This individual was subsequently paired with a randomly chosen male (=sex allele). Per sample of x mated females, the number of different sex alleles was determined. The values in the figures represent the mean and standard deviation of 100 repeats.

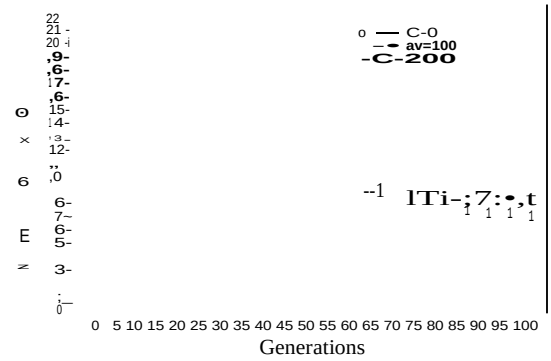

Fig. 4. Relationship between the mean number of sex alleles present in the population and the number of generations this population was maintained at 100 males and 100 females, when the number of offspring produced by each pair is fixed, or varies with a coefficient of variation (CV) of 100 or 200. Error bars represent the standard deviation around the mean. :dean and standard deviation are based on 100 replicates.

In the simulations to determine the loss of alleles in the laboratory, the initial population consisted of a random sample of mated females from the field population as described above. In the following (lab) generations, each female was allowed to produce 10 male and 10 female off spring. In these generations, diploid male offspring could be formed; however, diploid males were not allowed to participate in the matings that formed the basis for the next generation. For population sizes (P) in each generation, P haploid males and P females were chosen randomly, and subsequently paired randomly to form the parents for the next generation. Male and female forming a pair only participated in one mating.

For simulations in which there was variance in number of offspring per female, the set-up was essentially the same as in the previous simulation; however, now for each female a number of offspring was drawn form a normal distribution with a mean (m) and variance (v). For all simu-

lation a mean of 5 individuals and a standard deviation of either 0, 5 or 10 was used. When values for the offspring production of <0.5 were generated in the drawing from the normal distribution, the family size was rounded off to zero offspring.

Finally, in the simulations with a variance in the number of matings per male, the number of matings for each male was drawn from a Poisson distribution with a mean number of matings of either 1 or 2. When the total number of P matings was attained, no additional males were selected.

Discussion

Theoretically, single-locus sex determination substantially influences the sex ratio and the population growth of parasitoid species. Both factors affect the biological control potential of parasitoids. Although scant data exist on the distribution of this sex-determining mechanism among the Hymenoptera, what data exist suggest that single-locus sex determination is limited to some, if not all, Braconidae and Ichneumonidae (Table 1). Chalcidoidea seem to possess another sex-determining mechanism (Luck et al. 1992). A review of the literature shows that predictions based on single-locus sex determination-i.e., an increasing frequency of males in the offspring of laboratory populations and a low establishment rate of populations released as biological control agents-occurs in Braconidae and Ichneumonidae. This is not the case for Chalcidoidea. Although these results are weak evidence for this hypothesis, its consequences for the practice of biological control are substantial and obvious. Clearly, more data are needed to document the extent to which the single-locus sex determination occurs within Hymenoptera and the degree to which it contributes to biological control failures.

The problems caused by this sex-determining mechanism can be alleviated. Simulations indicate that only a small sample (20-30 mated females) includes most of the alleles present in a randomly mating population, provided that the individuals used to start the culture are unrelated. However, larger samples are always better. If the species is gregarious, the offspring from each parasitized host will probably be from a single-mated female. The geographic distribution of sex alleles in a species is unknown, but it is conceivable that some alleles occur only in certain areas. Combining samples from different geographic regions may enrich the pool of sex alleles. But there is a risk. Wasps from different areas may exhibit some crossing incompatibility (Mackauer 1969, Hoy & Cave 1988, Stouthamer 1989, Pinto et al. 1991).

Two approaches can be taken to maximize the diversity of different sex alleles during laboratory propagation and mass rearing: (1) the wasps can be maintained as one large population, or (2) they can be kept as a large number of isolated subpopulations. The first approach reduces the rate at which alleles are lost (Fig. 3). The second approach results in many alleles being lost in each subpopulation, but at least two sex alleles are retained. All the different alleles will likely be present in the amalgam of these subpopulations.

When large cultures are maintained, the following culture practices reduce the loss of alleles: promoting a rapid increase in the population size of a culture after it is initiated and maintaining it as large as possible thereafter, maximizing the number of pairs contributing offspring to the next generation (in the case where host material is limited, let each female parasitize only a few hosts, instead of a few females parasitizing most hosts, allowing each female to contribute the same number of male and female offspring to the next generation, excluding offspring of females that produce a low frequency of daughters from participating in the breeding pool of the next generation because, this likely indicates diploid male production, and fostering multiple mating in species that do so.

In the second approach, as many as possible small, isolated cultures should be maintained. However, this technique suffers from several disadvantages: a large proportion of hosts is wasted on the production of diploid males and in cases where the diploid males mate, a strong male-biased sex ratio is generated (Fig. 1), with the consequent potential for extinction of the culture. Inbreeding effects on other loci may also contribute to the demise of these cultures. When wasps are mass produced for field release, individuals from the separate cultures should be combined into one large culture. This minimizes the production of diploid males during the mass production and maximizes the number of sex alleles in the released population.

Even when a large number of sex alleles is released in the field, problems associated with sex determination may occur. First, field colonizations in which only a few wasps are released at any one location can result in the introduction of only a subsample of the sex alleles. Therefore, it is important to release many wasps per colonization site. Second, the dispersal of wasps from a colonization site also may generate problems related to sex determination. For instance, if a mated female disperses from the original release site and then oviposits, her offspring will likely sib mate and produce diploid males. Thus, the spatial aspects of the subsampling of sex alleles, together with the associated increased levels of inbreeding, slows the growth of a population. This should only be a temporary problem, as alleles are expected to spread evenly with time. To avoid this slowdown in population growth, releases can be made in a grid pattern.

The success of established populations with few sex alleles can be enhanced by importing and releasing additional alleles. In this case fewer individuals need to be released because the new sex alleles have a selective advantage.

Clearly, single-locus sex determination has the potential to impede biological control severely if appropriate precautions are not taken. Thus, elucidating the sex-determining mechanism in those species selected for biological control will likely increase the rate of success. Sex ratio problems in laboratory cultures should be reported and studied. Species with a single-locus sex determination should not be discarded a priori as potential biological control agents. With proper precautions, their mechanism of sex determination is not an impediment to successful biological control. Moreover, it may be worthwhile to reexamine some of the parasitoid species that have failed in the past and determine whether their failure was caused by low allelic diversity at the sex locus.

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