MICROPLITIS CROCEIPES (CRESSON)[1]: GENETIC CHARACTERIZATION AND DEVELOPING INSECTICIDE RESISTANT BIOTYPES[2]

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ABSTRACT

Microplitis croceipes (Cresson) is a promising biological control agent for *Heliothis virescens* (F.) and *Heliothis zea* (Boddie). Its utility in biological control programs may be enhanced if insecticide resistant strains are developed which can be utilized in augmentative releases. The efficacy for developing resistant *M. croceipes* strains is dependent on the genetic potential and variability which resides in the parasitoid genome. Investigations characterizing the extent of genetic variation using electrophoretic techniques reveals thirty-seven proteins, eight (21.6%) of which are segregating for two or more electromorphs. The average heterozygosity summed across all loci is 21.2 with an average per locus heterozygosity of 6.4. Variation in inbreeding is also found and is formulated as the "inbreeding effect index (I.E.I.)" which grows smaller as the potential for producing a 1:1 sex ratio also decreases. The index appears to vary independently of the pupal emergence rate which remains essentially unchanged. This confirms the expectation that inbreeding of lethals is not the cause of increasing male production associated with loss of inbreeding lines. It also suggests the sex determining locus is somehow involved in observed inbreeding effects. We conclude that, although allozymes indicate sufficient genetic variation exists, inbreeding at the sex determining locus may be a problem when selecting for insecticide resistant biotypes of *M. croceipes*.

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INTRODUCTION

The studies by Stadelbacher et al. (1984) and King et al. (1985) have focused much attention on *Microplitis croceipes* (Cresson) as a primary hymenopteran parasite attacking the bollworm, *Heliothis zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.). Apparently, *M. croceipes* is more effective than other parasites because the braconid parasitizes larger numbers of *Heliothis* spp. larvae in more species of host plants and is active over a longer seasonal period than other parasites. Hopper and King (1984a) have studied the feeding preference of different instars of different species of *Heliothis*, characterizing the effect *M. croceipes* has on feeding and movement of its parasitized hosts (Hopper and King 1984b). Other researchers working with host preference (Lewis 1970), host-seeking behavior (Nordlund and Lewis 1985, Powell and King 1984, Nordlund and Sauls 1981, Gross et al. 1975, Lewis and Jones 1971), rearing (Sauls et al. 1979, Lewis and Burton 1970), life history characteristics (Lewis and Snow 1971), and physiology (Jones and Lewis 1971) have developed much information on the biology of *M. croceipes* since the studies of Bryan et al. (1969).

Recent studies have focused on survival of the parasitoid exposed to different insecticide regimes (Bull et al. 1987, Katayama et al. 1987, Powell et al. 1986, Powell and Scott 1985) and even to the fungicide Maneb (Felton and Dahlman 1984). Common to the insecticide studies has been the finding that *M. croceipes* is somewhat tolerant to certain synthetic pyrethroids (SP), less so to several carbamates, and sensitive to organophosphates (OP) such as methyl parathion. These studies suggest that the increased importance of *M. croceipes* in acting as a control agent for *Heliothis* spp. may be due to the relatively recent advent and use of pyrethroid insecticides. These may have acted to reduce other competitors for the host. The tolerance of *M. croceipes* may have developed as a residual cross resistance to past applications of DDT (Bull et al. 1987). Multigenerational exposure to DDT affords a selection regime for physiological or biochemical detoxification traits. It confers tolerance to SP, a condition first noted to exist in house flies and ticks, where paralysis by DDT (called knockdown resistance or KDR) was related to resistance to pyrethroids (Plapp 1976).

Bull et al. (1987) have also investigated the biochemical basis for the observations concerning relative resistance to SP versus OP. Their evidence suggests that hydrolase (esterase) and glutathione-S-transferase (GSH-transferase) may be the primary detoxification systems in *M. croceipes* versus detoxification by the microsomal oxidase (MFO) system.

The latter is very active in *Heliothis* spp. Thus, Bull et al. (1987) have suggested that *M. croceipes* could have a selective advantage over its hosts wherever pyrethroids are used.

The above review suggests *M. croceipes* may be a prime candidate for development of insecticide resistant biotypes useful in IPM projects attempting to control *Heliothis* spp. The first insecticide type to consider developing a resistant strain too would be pyrethroids because of the residual resistance already present. The question what is the genetic potential available for such development naturally arises. We report here studies of allozymes and our attempts at inbreeding to develop homozygous lines. These studies give some insight into the extent and nature of genetic variability in *M. croceipes*.

MATERIALS AND METHODS

Samples of *M. croceipes* were obtained from the colony maintained by the USDA Southern Field Crops Insect Management Laboratory, Stoneville, MS. In these colonies, hundreds of females may oviposit each generation. The colony is supplemented in late July-early August with wild individuals to maintain genetic variability and to lower the influence of genetic drift. *H. virescens* is used as the 3rd-instar larval host.

The electrophoretic techniques, histochemical methods, procedures and equipment described in Steiner and Joslyn (1979) were used to examine the 37 protein gene loci shown in Table 1. Inheritance studies of those found to be segregating for two or more alleles will be reported elsewhere. Here, we accept the definition that a locus is polymorphic if the most common allele is found 99% of the time or less. In our studies, *Drosophila melanogaster* (Meigen) was used as a control to verify enzyme activity. Mobility of any isozyme band on a starch gel was taken relative to the most commonly occurring isozyme band at that locus. This approach meant that regardless of the buffer system used for separating the bands, an internal control was assured. Our studies over the last two years have indicated that in the polymorphic systems, frequencies of the most common allele remain stable unless great population density perturbation is experienced.

The most commonly occurring allele was given the arbitrary assignment *100/100* in the homozygous state. Depending on the running conditions for separating the proteins, faster or slower migrating protein bands are named by adding or subracting their relative difference in migration distance in *mm* from the common (*100*) type. Thus, in Fig. 1 for *PGI*, (reading left to right) sample no. 1 is *100/100*, sample no. 2 is *95/100*, samples 3 - 6 are *100/100*, sample 7 is *95/95*, samples 8 and 9

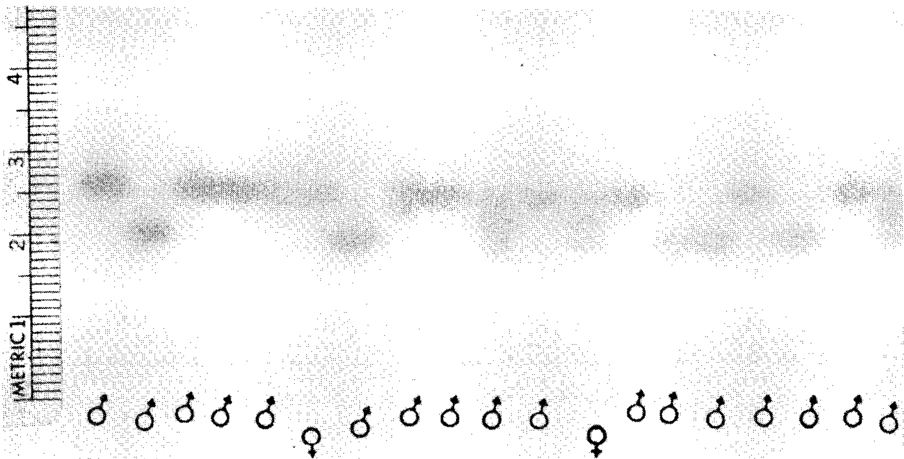

FIG. 1. Starch gel stained for the enzyme PGI (see text) of *M. croceipes*. Note the three-banded heterozygotes indicating the PGI quaternary structure is dimeric.

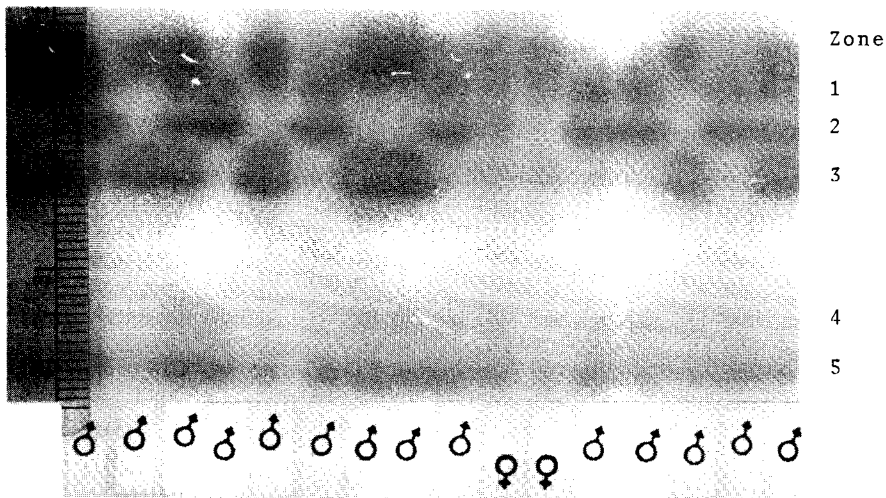

FIG. 2. Starch gel stained for esterases (see text) of *M. croceipes*. Major variation can be seen in the second zone from the top, termed EST-2. See text for additional explanation.

are 100/100, samples 10 and 12 are 95/100, samples 11 and 13 are 100/100, samples 14, 15 and 17 are 95/95, samples 16 and 18 are 100/100, and sample 19 is 95/100. We use this nomenclature system throughout this paper.

TABLE 1. Proteins, Their Enzyme Classification Number (E.C.), Their Abbreviation (Parentheses), the Number of Loci Encoding Them, and the Buffer System They Can Be Separated on, in the Parasitoid *Microplitis croceipes*[a].

Protein[b,c] System	Enzyme Classification Number		Number of Loci coded	Buffer
Acid Phospbatase	3.1.3.2	(ACPH)	NR	LiOH
Alcohol DH	1.1.1.1	(ADH)	NR	or NaOH
Aldehyde Oxidase	1.2.3.1	(ALDOX)	1	"
Alkaline Phosphatase	3.1.3.1	(APH)	NR	"
Amylase (acrylamide only)	3.2.1.1	(AMY)	1	"
Esterase	3.1.1.1	(EST)	5	"
General Proteins	----	(CBB)	NR	"
Glutamate Oxaloacetate Transaminase	2.6.1.1	(GOT)	2	"
Leucine Aminopeptidase	3.4.1.1	(LAP)	4	"
Malic Enzyme	1.1.1.40	(ME)	1	"
Peptidase	3.4.1.1	(PEP)	3	"
Phosphoglucomutase	2.7.5.1	(PGM)	2	"
Phosphoglucose Isomerase	5.3.1.9	(PGI)	1	"
Superoxide Dismutase	1.15.1.1	(SOD)	1	"
Triosephosphate Isomerase	5.3.1.1	(TPI)	3	"
Glucose-6-phosphate DH	1.1.1.49	(G6PDH)	1	CA-8
alpha-Glycerophosphate DH	1.1.1.8	(a-GPDH)	3	"
Isocitrate DH	1.1.1.42	(IDH)	1	"
Hexokinase	2.7.1.1	(HK)	2	"
6-phosphogluconate DH	1.1.1.44	(6-PGDH)	1	"
Adenylate Kinase	2.7.4.3	(ADK)	1	"
Aldolase	4.1.2.13	(ALD)	1	"
Gly.-3-ph. DH[d]	1.2.1.12	(G3PDH)	1	"
Malic DH	1.1.1.37	(MDH)	2	"

[a] Number of samples equals 60 or greater in all cases.
[b] See Steiner and Joslyn (1979) for a description of equipment, stain techniques and buffer systems.
[c] DH = Dehydrogenase. NR = No results.
[d] Glyceraldehyde-3-phosphate Dehydrogenase.

Inbreeding using sib x sib matings was initiated in order to make homozygous lines fixed for a single allele found at the polymorphic loci. Copulation was observed between individuals which were selected at random from the available generation. Six such matings were done for each of 27 initial lines, unless the female number was too low, in which case only the available females were used. The female parasitoid was then allowed to oviposit at random into 50 larval hosts (with the expectation of 50% survival). When parasitized larval behavior (lack of pupation) was observed in the host _H. virescens_, the parents were frozen and stored for electrophoresis at a later date. These studies continued through five generations when inbreeding depression became a serious problem. In the sixth generation, females of the inbred lines were outbred to males of lines which had most recently tested similar in electrophoretic genotype. After six generations, all lines were discontinued for lack of manpower and the increasing percentage of all-male lines.

The data collected during inbreeding included the male:female sex ratio, the percent of isolines producing all males, and the pupal emergence rate. From the first two statistics, the inbreeding effect index (I.E.I.) was formulated to provide a measure of the deviation from a 1:1 sex ratio. In this case, we let

1 = expected value = a 1:1 sex ratio.

Then:

1 - proportion of isolines producing all males

= a measure of the deviation from a 1:1 ratio.

To adjust for the observed sex ratio, we have:

I.E.I. = 1 - proportion of lines producing all males/
observed sex ratio

= potential for producing a 1:1 sex ratio.

When the sex ratio is 1:1, we can let the expected value of 50% males + 50% females = 100% of offspring be met and the formula then reduces to:

I.E.I. =1 - proportion of lines producing all males/1

=1 - proportion of lines producing all males.

Obviously, if the 1:1 sex ratio occurs, then the last term (1 - proportion of lines producing all males) reduces to one and our potential expected value is met.

RESULTS

Polymorphic proteins include two esterases (EST-2 and EST-4), phosphoglucose isomerase (PGI), 6-phosphogluconate dehydrogenase (6-PGDH), glutamate oxaloacetate transaminase-1 (GOT-1), hexokinase (HK), malate dehydrogenase-1 (MDH-1) and phosphoglucomutase-1 (PGM-1). Analysis of over 1,000 individuals in the past two years has shown no variation at other loci listed in Table 1.

Four loci (PGI, GOT-1, 6-PGDH, and EST-2) had the most common allele occurring at less than 92% frequency. Consistently polymorphic, these loci were used in the selection and inbreeding experiments discussed below.

Table 2 gives a brief description of the variable loci along with the electrophoretic conditions to separate them. The migration distance has relevance only with respect to the running conditions given.

In *M. croceipes*, the most interesting variation amongst the isozymes is associated with the esterases (Fig. 2). Five aryl-esterase zones can be observed on

TABLE 2. Characteristics of Variable Protein Loci in the Entomophagous Parasitoid, *Microplitis croceipes*.

Locus	Structure	Migration Distance of standard from origin Distance (mm)	Buffer System[a,b] Description		
				gel	electrode
PGI	dimer	23.0	LiOH:	pH=8.5	8.3
				TB=6.2g/l	---
				CA=1.6g/l	---
				Li= ---	1.2g/l
				BA= ---	11.9g/l
GOT-1	dimer	18.0	"	" "	" "
EST-2	?	46.0	"	" "	" "
EST-4	monomer	20.0	"	" "	" "
PGM-1	monomer	22.0	"	" "	" "
6-PGDH	monomer	19.0	CA-8:	pH=8.4	8.2
				CA=1.7g/l	66.0g/l
				TB=9.0g/l	166.4g/l
			dilute anode tray		1:3
			dilute cathode tray		1:2
HK-2	monomer	15.0	"	" "	" "
MDH-1	dimer	24.0	"	" "	" "

[a] The gel uses Electrostarch at a concentration of 13%.
[b] TB=Trizma Base; CA=Citric Acid monohydrate; Li= Lithium Hydroxide; and BA=Boric Acid. See Table 1 for locus abbreviations.

the gel at 12, 20, 25, 45 and 53 mm from the origin. We have named these EST-5, EST-4, EST-3, EST-2 and EST-1 respectively, with EST-5 the slowest migrating zone found closest to the origin. Zone EST-4 has a rare allozyme occurring at 3.7% or less in frequency. Zone EST-2 is clearly polymorphic, segregating for two allozymes. All other esterases seem to show no genetic variation, although EST-1 is not clearly definable at all times and sometimes the variation observed for this locus is correlated with zone EST-2. We have chosen to ignore variation at EST-1 until we can better resolve and understand this system.

We have concentrated our attention on the variation at EST-2. This variation is unusual in that the slower-migrating allozyme appears to be made up of two bands, while the faster migrating allozyme consists of only one band (Fig. 2; from the left, compare males 1 and 4 with males 2 and 5, the latter having the two slow bands present). Heterozygotes do occur in this system (samples no. 3 and 16). In males, all three bands are present. In the females, only two bands are often present, the middle band disappearing. Notice from the figure that where two bands are present in the EST-2 slow phenotype, then zone EST-1 tends to have an additional, faster band as well. This is the first case we have seen in insects where additional bands are seen in one phenotype but not the alternative phenotypic state. Harris and Hopkinson (1976) demonstrated that two faint bands may be seen in alternative phenotypic states in human esterases and other allozymes, which often are masked by the hybrid band in the heterozygote. These additional bands are believed to be secondary forms perhaps due to isomeric formation or differential folding of the protein chain altering the quaternary structure without affecting activity, thus being independent of the hybrid band in dimeric protein systems. However, in the present case, the sex related effect where males but not females display two bands for the slow allele of EST-2 suggests sex determined or sex limited effects.

Generally, females tend to be weaker in activity at EST-2 when compared to males (Fig. 2, samples no. 10 and 11 from the left). In some isolines undergoing selection to fix the esterase alleles, activity became nonexistent, indicating selection favoring a null allele or an allele at a second locus which reduced esterase activity. We can not say which interpretation is correct, but we favor the latter because we detected non-Mendelian ratios in some inbred lines regarding presence or absence of enzyme activity. Non-Mendelian ratios were also detected in strong-versus weak-staining activity in female heterozygotes.

Table 3 gives an estimate of how heterozygous the genome of *M. croceipes* is as determined from the observed

gene frequencies given for diploid females only. The estimate takes into acount the nature of sexual reproduction in this species. We can calculate two estimates (Spiess 1977). Average observed heterozygosity is the number of all heterozygotes observed summed across all gene loci divided by the number of loci and is 21.2 with an average within locus heterozygosity of 6.4. The second is an estimate of the number of polymorphic gene loci, assuming the most common allele occurs 99% of the time or less. Under this criterion, 21.6% of gene loci are segregating for two or more alleles. These estimates should be considered high for Hymenoptera but may be average for parasitoids since Unruh et al. (1983) found equivalent variation in _Aphidius ervi_ (Haliday) (but see Graur 1985, Owen 1985, and Metcalf et al. 1975 for lower estimates). We can conclude sufficient genetic variation in soluble proteins is present in _M. croceipes_ to make it a desirable field and laboratory insect for demonstrating genetics of parasitoid haplo-diploid systems.

Results of the inbreeding experiments, aimed at developing lines fixed for alternative alleles at the loci

TABLE 3. Protein Gene Loci Demonstrating Variation in Females of a Colony of _Microplitis croceipes_.[a]

Locus	Year	No. of Alleles	Frequency of allele 90	95	100	105	Null
PGI	1984	2		0.299	0.701		
	1985	2		0.222	0.778		
GOT-1	1985	2		0.159	0.841		
6-PGDH	1985	2			0.911	0.089	
HK-2	1985	2			0.962	0.038	
EST-2	1984	3	0.010	0.227	0.705		0.058
	1985	3		0.107	0.883		0.040
EST-4	1985	2			0.963	0.037	
MDH-1	1985	2			0.983	0.017	
PGM-1	1985	3		0.016	0.968	0.016	

[a] The number analyzed is 60 or greater for each locus. Equilibrium gene frequencies have not been calculated due to the mode of reproduction.

PGI, *EST-2*, *6-PGDH* and *GOT-1*, are summarized in Table 4. The data shows a continual rise in the sex ratio. By the generation five, the male:female ratio is 6.94:1. This is paralleled by an increase in the percent of lines that go extinct by producing only males. Note the I.E.I. value also grows smaller as inbreeding produces more and more all-male lines and the sex ratio increases. The I.E.I. clearly shows that the potential to produce a 1:1 sex ratio is deteriorating across all isolines as inbreeding continues.

DISCUSSION

Since esterases are very often associated with insecticide resistance, the above observations raise interesting questions. We are currently investigating whether there are any associations between the

TABLE 4. Changes in Sex Ratio, Percent Gender, and the Associated Inbreeding Effect Index (I.E.I.) with Generation in Inbred *Microplitis croceipes*.[a]

No. of Isolines Tested	N	Generation	Sex Ratio[b]	% Lines All Male	I.E.I.	Pupal Emergence Rate
27	513	F_2	2.89	25.9	.256	88.4
32[c]	251	F_3	3.40	37.5	.184	74.3
23	347	F_4	3.24	56.5	.134	86.8
41	255	F_5	6.94	70.7	.042	DNC[e]
52[d]	862	F_6	4.22	21.2	.187	DNC

[a] Parental pairs of each generation were observed for copulation but females were not examined for sperm transfer. From 1 - 6% of lines in each generation had no offspring. See text for calculation of inbreeding effect index.
[b] Males to females.
[c] Additional lines added as sublines of sibmatings from original lines.
[d] Outbreeding program between isolines initiated.
[c] DNC = Data not collected.

esterase-activity mutants observed on the gels and longevity under pyrethroid selection, or between the different allozyme types and pyrethroid selection.

In Hymenoptera, unfertilized eggs normally develop into haploid males while fertilized eggs develop into diploid females. Diploid males are not expected and indeed may be sterile because the sperm of diploid males is too large to fit through the egg micropyle (Crozier 1977). The sporadic occurrence of heterozygous males at the polymorphic loci was a surprise since _M. croceipes_ uses a haplo-diploid system of reproduction (males are hemizygous). This finding suggests that diploid males can occur in this braconid, a situation also noticed in _Habrobracon juglandis_ (Ashmead) some years ago (Martin 1947), in the fire ant _Solenopsis invicta_ (Buren) (Ross and Fletcher 1985), and in at least 12 other hymenopteran species (Crozier 1977). At least one other report used allozyme variation to detect diploid males (Hedderwick et al. 1985).

Horn (1944) and Whiting (1939) established that sex determination was under the control of a single gene locus with multiple alleles present in _Habrobracon_ sp. The number of alleles can have an affect on the sex ratio, as demonstrated in Table 5. Two alleles would be the minimum number possible to have male and female sexes, but evolutionary theory predicts selection will reduce gamete wastage, and so a multiple allele system should be favored similar to that proposed by Ross and Fletcher (1985). Thus, the more alleles present the more likely that fertile matings producing both sexes will result. Table 5 clearly indicates how the percent of males declines as the expected number of crossing types increases with the increase in sex allele number. This leads to a decrease in production of diploid males, an increase in the number of fertile haploid males (from 66.7% of all males for models having 2 sex alleles, to 83.3% with 5 sex alleles) and normalization of the sex ratio.

Table 5 suggests the answer to the inbreeding problem leading to production of all-males in the selected isolines of Table 4. The fewer the number of sex alleles, the higher the number of sterile diploid males and the lower the number of males which can effectively mate (column 6). Since inbreeding sometimes involves crosses with sterile diploid males, the sex locus demonstrates the consequences. Homozygosity will result within a few generations of inbreeding, where choice of mates are affected by chance and small available numbers.

One might expect, in haplo-diploid mating systems where sex is determined by a single gene with three or more alleles, inbreeding or sibship matings might not be favored if the female is always the heterozygous sex. This seems to be the case where investigations have been done (Crozier 1977). Evolution, in this case, might very

well involve development of *cis* and *trans* relationships between genes and their modifiers which effect sex determination. This could lead to fixation on opposing chromosomes of the relevant genes in such a way that recombination would become very rare. Genetic coadaptation might then arise along each type of chromosome. Indeed, Biemont and Bouletreau (1980) have found evidence in the parasitoid wasp *Cothonaspis boulardi* (Barbotin, Carton and Kelner Pillault) suggesting coadaptation might involve the whole genome in order to reduce any effects due to mutation and segregation load. Regardless, we would suggest that coadaptation of parasitoid wasp genomes might involve the chromosome carrying the sex determining locus more than any other.

It is now clear that although genetic variability appears relatively high in *M. croceipes*, inbreeding to establish homozygous lines for gene mapping and genetic manipulation will be a problem. Inbreeding may lead to increased production of diploid males as *cis* and *trans* relationships begin to break down and increase the opportunity for recombination to take place.

One way around this problem is to follow each generation of selection with a generation of outbreeding. This would be a "hit-or-miss" affair, since we have no way of determining differences between carriers of different sex alleles at present (for example, the outbreeding sex may by chance have the same sex determining allele as the inbred line). It may be possible, using mtDNA restriction length polymorphism analyses, to determine cytological markers which would mark our different isolines. This would give us clues of how to perform the desired crosses so as to inbreed the genetic background and fix the genes desired while simultaneously outbreeding the sex determining locus. An alternative approach also exists which would be dependent on molecular genetic techniques. The recent discovery that amplified esterase gene systems play a role in insecticide resistance in mosquitoes and houseflies (Hyrien and Buttin 1986, Mouches et al. 1986) has important implications for strain development. Gene amplification may be a major mechanism by which insects develop resistance, and deserves investigation in planthoppers (Hasui and Ozaki 1984) and Egyptian cotton leafworm (Riskallah 1983) besides Diptera. Indeed, De Jersey et al. (1985) found esterase activity to be associated with serine dependant esterases, active around a pH of 5.5 - 6.0, which use alpha-napthyl acetate for a substrate. Since these are a major esterase component in most insects, implications for development of insecticide resistance are obvious.

It is well-known insecticide resistance responds to genetic selection. Recent attempts have been successful in beneficial entomophagous mites (Strickler and Croft 1982, Hoy and Knop 1981), the green lacewing (Grafton-Cardwell and Hoy 1981) and for DDT resistance in para-

sitoids (Pielou and Glasser 1952). Although the mite *Amblyseius fallacis* (Garman) demonstrated a 60-fold increase

TABLE 5. Mating Models for Effect of Increasing Sex Alleles on Effective Sex Ratio in a Haplo-Diploid Mating System Assuming Females Are Always the Heterozygous Sex, Diploid Males Are Sterile, and Fertilization Occurs Half the Time.

No. of Alleles	Expected Female X Male Crosses[a]	Overall Male:Female Sex Ratio	% Males	Effective Sex Ratio	% Effective Males	% Diploid Males
2	S1S2 X S1 S1S2 X S2 (4:2:2)	3:1	75.0	2:1	66.7	33.3
3	S1S2 X S1 S1S2 X S2 S1S2 X S3 S1S3 X S1, S2S3 X S1 S1S3 X S2, S2S3 X S2 S1S3 X S3, S2S3 X S3 (18:6:12)	2:1	66.7	1.5:1	75.0	25.0
4	S1S2 X S1, S1S4 X S1, S3S4 X S1 S1S2 X S2, S1S4 X S2, S3S4 X S2 S1S2 X S3, S1S4 X S3, S3S4 X S3 S1S2 X S4, S1S4 X S4, S3S4 X S4 S1S3 X S1, S2S3 X S1, S2S4 X S1 S1S3 X S2, S2S3 X S2, S2S4 X S2 S1S3 X S3, S2S3 X S3, S2S4 X S3 S1S3 X S4, S2S3 X S4, S2S4 X S4 (48:12:36)	1.67:1	62.5	1.33:1	80.0	20.0
5	S1S2 X S1, S1S3 X S1, S1S4 X S1, S1S5 X S1, S2S3 X S1 S1S2 X S2, S1S3 X S2, S1S4 X S2, S1S5 X S2, S2S3 X S2 S1S2 X S3, S1S3 X S3, S1S4 X S3, S1S5 X S3, S2S3 X S3 S1S2 X S4, S1S3 X S4, S1S4 X S4, S1S5 X S4, S2S3 X S4 S1S2 X S5, S1S3 X S5, S1S4 X S5, S1S5 X S5, S2S3 X S5 S2S4 X S1, S2S5 X S1, S3S4 X S1, S3S5 X S1, S4S5 X S1 S2S4 X S2, S2S5 X S2, S3S4 X S2, S3S5 X S2, S4S5 X S2 S2S4 X S3, S2S5 X S3, S3S4 X S3, S3S5 X S3, S4S5 X S3 S2S4 X S4, S2S5 X S4, S3S4 X S4, S3S5 X S4, S4S5 X S4 S2S4 X S5, S2S5 X S5, S3S4 X S5, S3S5 X S5, S4S5 X S5 (100:20:80)	1.5:1	60.0	1.25:1	83.3	16.7

[a] S1=sex allele 1, S2 = sex allele 2, S3 = sex allele 3, etc. Summary ratios of haploid males:diploid males:females are in parentheses.

in resistance in 12 generations, the above species have shown at best moderate responses, usually in the 5 to 10-fold range. Selection of this type, where each generation is exposed to continually higher and higher doses of insecticide with survivors forming the breeding nucleus for the next generation, may take 1 - 3 years to complete.

Gene amplification can now be engineered in yeasts, suggesting it is only a matter of time before it is attempted in insects. This approach would enable a resistant strain to be developed to specific insecticides within at most three generations (the engineered generation, a generation to select the engineered type, and a generation to build up sufficient numbers to work with). This approach may be most inviting where the beneficial entomophage has a complicated biology such as the inbreeding effects noted here which hinder genetic selection techniques. Under selection, even with sufficient amounts of genetic variation, the time it might take to develop insecticide resistant strains may be longer than the time it takes for pest field populations to develop resistance to the insecticide of interest. In this case, genetic engineering techniques offer a quick way to bypass complicating problems.

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