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HETEROZYGOSITY AND EFFECTIVE SIZE IN LABORATORY POPULATIONS OF *APHIDIUS ERVI* [*HYM. : APHIDIIDAE*]

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Decline of allozyme variability in 7 laboratory populations of *Aphidius ervi* Haliday demonstrates that the effective population size is approximately one half the number of individuals used to renew the cultures each generation. Unequal reproductive contribution by individual females to subsequent generations is the most likely cause for this phenomenon.

Intraspecific genetic variation may be one measure of the quality of natural enemy populations imported for classical biological control. Variation in such biologically important attributes as diapause (**Hoy**, 1978), temperature tolerance (**Flanders**, 1931), sex ratio (**Skinner**, 1982), host preference (**Baker**, 1976) and pheromone components (**Klun & Maini**, 1979 ; **Lanier** *et al.*, 1980) is well documented in insects and is often associated with geographically widespread or disjunct populations.

Importing natural enemy populations with potentially new and useful traits and preserving these traits through the rigors of importation, quarantine and release are persistant challenges for classical biological control. To further improve our methods in these endeavors, protein electrophoresis was employed to document the fate of genetic variation in natural enemy populations subjected to typical importation and laboratory culture procedures. Even though much of the genetic variation detected by electrophoresis may be effectively neutral, it does provide a reliable measure of genetic drift which may act to reduce genetic variation in both adaptive and neutral traits.

In a preliminary study we examined 12, 1 to 5 year old, laboratory populations of *Aphidius ervi* Haliday at 10 to 25 enzyme loci and found very low levels of variation. Most populations were homozygous at all loci, 2 were heterozygous at 1 locus, 1 was heterozygous at 2 loci and 1 was heterozygous at 3 loci. These levels of variation are lower than that reported for other Hymenoptera (**Pamilo** *et al.*, 1978). These populations were collected from several geographically distinct sources (**Gonzáles** *et al.*, 1978). In contrast, when we examined the 1st generation of an *A. ervi* population from Japan, 10 of 27 loci were polymorphic with an average heterozygosity of 8 % per locus per individual. We suspected that genetic drift in the older laboratory populations accounted for this discrepancy. Other, newly collected field populations of *A. ervi* also displayed high levels of allozyme variability when compared to populations maintained in the laboratory for long periods.

The present study was undertaken to evaluate the importance of genetic drift in our laboratory populations. We subcultured several populations of the polymorphic biotype, *A. ervi* (from Morioka, Japan ; parasitizing *Acyrthosiphon pisum* (Harris) feeding on *Medicago sativa* L.) (**Gonzalez** *et al.*, 1979), and monitored the change in electrophoretically detectable variation through time. Herein we describe the decline of allozyme variation in 7 laboratory populations maintained at 4 different population sizes in 3 different temperature regimes. The genetic models for the inheritance of 6 of the 8 polymorphic loci monitored in this study are presented and polymorphism among other populations of *A. ervi* is described. The results are discussed in light of theory and the practical constraints of biological control.

MATERIALS AND METHODS

LABORATORY POPULATIONS

In November 1979, we received a shipment of 73 ♀♀ and 30 ♂♂ *A. ervi* from Morioka, Japan. Sixty-four ♀♀ survived to produce progeny which gave rise to the cultured populations depicted in figure 1. These parasites and all subsequent generations were allowed to oviposit

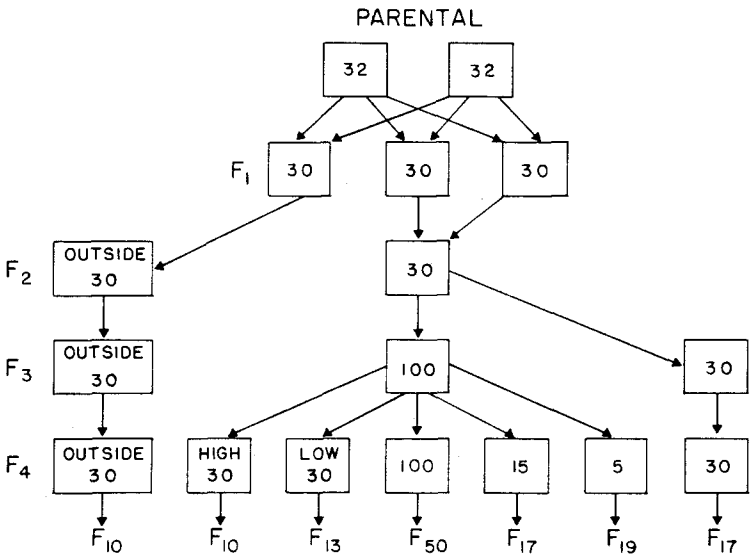

Fig. 1. Summary of the population subculturing that occurred to produce and maintain 7 populations of *A. ervi* from 64 field collected, laboratory mated, ♀♀. The numbers in each box represent the number of mated ♀♀ that were placed in a cage with fresh hosts at the beginning of each generation. Each generation, F_X, reflects the number of generations from the parental field collected individuals.

and develop in pea aphid, *A. pisum*, reared on broad bean, *Vicia faba* L. Sizes of the *A. ervi* cultures (5, 15, 30 and 100 ♀♀/cage) reflect the number of ♀ wasps that were aspirated from cages during peak emergence and transferred to clean cages with fresh aphid hosts each generation. All ♀♀ were assumed to be mated when they were transferred. Two pots of aphid-infested

broad bean plants were used to maintain the 5 and 15 ♀ populations. Four and sixteen pots were used for 30 and 100 ♀ populations, respectively. Each pot contained more than 1000 aphids of size classes suitable for parasitization. Four populations (5, 15, 30 and 100 ♀ lines) were maintained under insectary conditions of 22 ± 1 °C with 50 ± 10 % RH. Three other populations were initiated and maintained with 30 ♀♀ *A. ervi* each. The high temperature population (High) experienced a 16 ± 1 °C to 36 ± 6 °C daily temperature cycle. The low temperature population (Low) experienced a 2 ± 1 °C to 22 ± 2 °C daily cycle. The last culture was maintained out of doors (Outside) and experienced shade temperatures which ranged from 7 to 31 °C. Humidity was uncontrolled and generation time varied with temperature.

Samples for electrophoretic assay were taken every other generation when the 5, 15, 30 and 100 ♀ cultures were changed. The High, Low and Outside cultures were sampled on their 5th and final generations (see fig. 1). The sampled wasps were stored in liquid nitrogen.

Single pairs of *A. ervi* were observed to mate and the ♀ allowed to oviposit for 24 h. The parents were frozen as were their progeny upon emergence 2 weeks later. Electrophoretic assay of these individuals established the genetic basis of the allozyme variation for the loci considered in our study.

Finally, the following experiment was conducted to discover the cause(s) of the genetic drift that was observed. Ten, presumably mated *Aphidius ervi* ♀♀ were aspirated from each of 5 separate cultures for 3 consecutive days beginning with the 1st day of emergence. Individual ♀♀ were placed in a brown paper bag containing a potted fava bean plant infested with pea aphid and the bag was secured. Progeny were allowed to emerge and die in the bag before they were removed, counted and sexed.

ELECTROPHORESIS

Standard horizontal starch gel electrophoresis techniques were employed (**Bush & Huettel** (1) ; **Selander** *et al.*, 1971). Buffer recipes were from the sources given in table 1 with 2 exceptions, both of which were modifications from **Selander** *et al.*, (1971) : (1) Tris citrate III (TC3) (bridge, 0.57 M tris, 0.157 M citrate (monohydrate), 0.001 M EDTA, pH 7.7 ; gel, 0.0248 M tris, 0.0059 M citrate, 0.5 mM EDTA, pH 7.7) ; and (2) Lithium borate (L10) (bridge, 0.24 M lithium hydroxide, 0.279 M borate. pH 7.55 ; gel) 1 part bridge plus 9 parts 0.0193 M Tris, 0.005 M citrate, pH 7.7). These 2 buffer systems provided satisfactory results with the 8 enzyme systems assayed for the cage studies.

Individual wasps were homogenized in 15 µl of 0.1 M tris-HCl containing 0.26 mM NADP and 0.32 mM NAD. This yields enough raw homogenate to saturate two, 3 by 12 mm. filter paper wicks (Whatman # 1) which can be loaded into different gels.

RESULTS

ELECTROPHORESIS

The loci examined in *Aphidius ervi* from Morioka, Japan are represented in table 1 with their abbreviated names, the buffer systems employed for them, and the number of alleles per locus with enzyme subunits, where applicable. Ten of the 27 loci were polymorphic and 8 of these were of sufficient quality and reliability to assay for the population cage studies. More than 2 alleles/locus were observed in both esterase loci, malic enzyme and fumarase ; however, calculations are based on pooling mobility classes to yield only 2 per locus.

(1) **G.L. Bush & R.N. Huettel.** 1972. Starch gel electrophoresis of tephritid proteins : A manual of techniques. IBP working group on fruit flies. Unpublished manuscript. 56 pp.

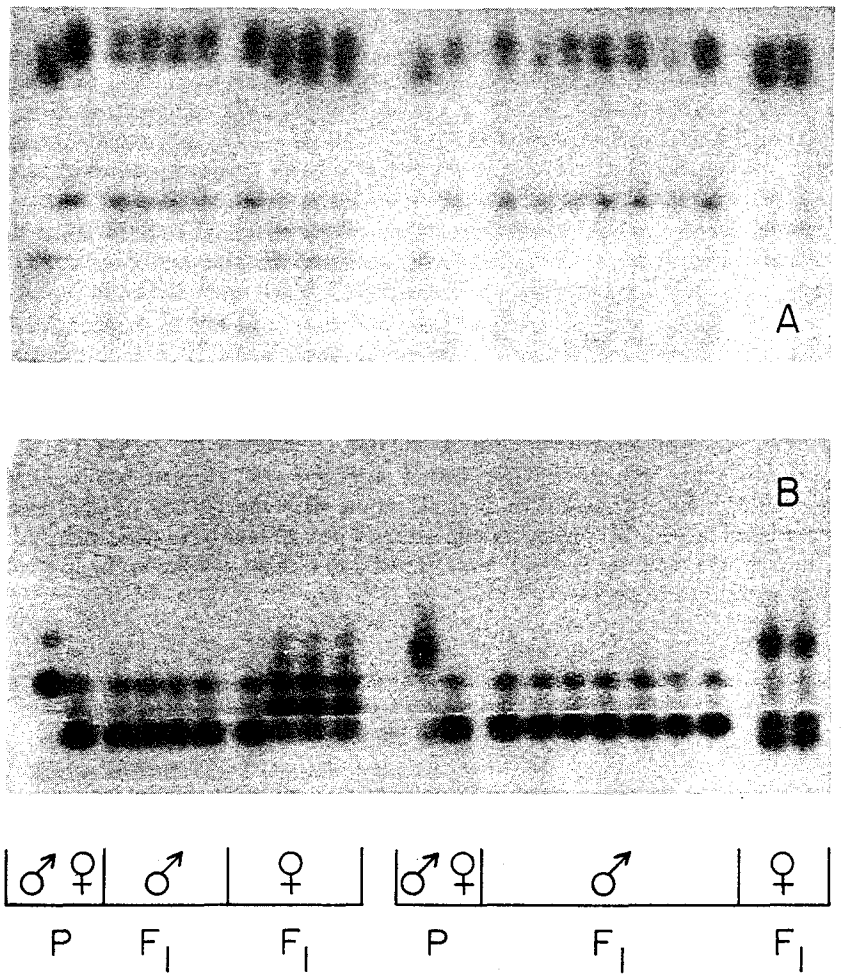

Fig. 2. Examples of electrophoretic assays of isolated mated pairs of *Aphidius ervi* and their progeny. Displayed are, from left to right on the gel, the phenotypes of a ♂ (Morioka, Japan), his mate (Beit Shéan, Israel), 5 ♂ progeny and 3 ♀ progeny, another ♂ (Morioka, Japan), his mate (Beit Shéan, Israel), 7 ♂ progeny and 2 ♀ progeny. 2A) Esterase I (most distal from point of insertion into gel) at the top behaves as a monomer but with the peculiarity that each electromorph displays 2 bands. Depending on the separation (run time, pH and molarity), heterozygotes will show 3 (4 bands but 2 overlapping) or 4 bands. Esterase II, the mobility class in the center of the gel, displays a typical dimeric enzyme banding pattern. A 3rd Esterase electromorph is apparent at the bottom of the gel but has poor resolution and was not considered. 2B) Phosphoglucose isomerase : the heterozygote phenotypes of the ♀ progeny in the 1st cross (same as 2A) is triple banded with the heteropolymer between the homozygote bands of their parents, indicating a dimeric enzyme. In the 2nd cross there is a triple banded phenotype in the ♀ progeny but the heteropolymer is slower than either of the parental homozygote phenotypes and occurs only when the fast allele (homozygous in the mother) is in heterozygous condition.

TABLE 1

Enzyme systems surveyed in Aphidius ervi *population from Japan. Included are the buffer systems employed in the survey, the number of alleles per locus and the subunit structure*

Enzyme locus	Abbreviations	Buffers used[a]	Alleles/locus	Subunit
Aconitase[c]	Acon	TC3	2	M
Adenylate Kinase	AK	Phos, JRP	1	–
Aldolase	ALD	TVB, TC3, TM		–
Alkaline Phosphatase[e]	ALP	Pou, TC3	1	–
Catalase	CAT	TC3	1	–
Diaphorase[e]	DIA	S5, TC3, Pou	1	–
Esterase I	EST I	TC3, Lio, Pou, Bor	4	M
Esterase II	Est II	Lio, Pou, Bor	3	D
Fructose 1, diPhosphate Dh[b,e]	F16Dh	S5, TC3	1	–
Fumerase[e]	FUM	TC3, S5	3	–
Galactose 6-Phosphate Dh[b,e]	GAL 6	Phos	1	–
Glucose 6-Phosphate Dh[b]	G6PDh	Hist, Phos, TC3	1	–
Glyceraldelyde-3-Phosphate Dh	G3PDh	TC3, S5	1	–
a-Glycerol Phosphate Dh I	α-GPI	TC3, S5, JRP, Phos	1	–
a-Glycerol Phosphate Dh II[e]	α-GP II	TC3, S5, JRP, Phos	1	–
Glutamate-oxaloacetate Transaminase	GOT	S1, Pou	1	–
Hexokinase I	HK I	JRP, Phos, TC3, S5, Lio	1	–
Hexokinase II	HK II	JRP, Phos, TC3, S5, Lio	1	–
Isocitrate Dh	IDH	S5, TC3, Lio	2	D
Leucine Amino Peptidase	LAP	S5	1	–
Malate Dh I	MDh I	TM, S5, TC3, Lio	1	–
Malate Dh II	MDh II	TM, S5, TC3	1	–
Malic Enzyme[e]	ME	TM, S5, TC3, Lio	3	–
6-Phosphogluconate Dh[b]	6-PGDh	S5, TC3	2	D
Phosphoglucose Isomerase[d]	PGI	Lio, Pou, TVB	2	–
Phosphoglucose Mutase	PGM	TC3, S5, TM	1	–
Triosephosphate Isomerase	TPI	S5, TC3	1	–

[a] TM (= Tris maleate), S1 (= Tris HCl), S5 (= Tris-Citrate II) and Pou (= Poulik's), from Selander *et al.*, 1971. TVB (= Tris-Versene-Borate, pH 9.0), Phos (= Phosphate, pH 7.0) and Bor (= Borate, pH 8.0) of Shaw & Prassad, 1970. Hist (= Histidine, pH 7.0) of Harris & Hopkinson, 1976. JRP (= Tris-Citrate) of Ayala *et al.*, 1972.

[b] NADP required in cathodal buffer and gel.

[c] NAD required in cathode buffer and gel.

[d] PGI does not appear polymorphic except on borate containing buffers.

[e] Resolution of discrete mobility classes is sometimes questionable in these systems.

The number of subunits of several loci is demonstrable. Esterase II, IDH, and 6-PGDh display 1:2:1 stain intensity patterns in heterozygotes, typical of dimeric enzyme structure. Crosses showing the inheritance for EST II are given in figure 2A. PGI also behaves as a dimer, except the heteropolymer is the slowest moving band in heterozygotes. This aberrant phenotype is clearly heritable (fig. 2B) but could only be visualized on borate-containing buffers. The slow heteropolymer occurs whenever the fast allele from the Morioka population is in heterozygous condition (tested against alleles from several biotypes). Unfortunately, this allele has been lost from our cultures, precluding further study. Other biotypes of *A. ervi* display considerable variation at the PGI locus, all with typical dimeric behavior (i.e. : the heteropolymer is of intermediate mobility). Aconitase displays 2 bands in heterozygotes and single bands in homozygotes indicating a monomer. EST I also behaves as a monomer but each allele produces a double band ; homozygotes display 2 bands, heterozygotes 4 (or 3 when bands overlap ; see figure 2A). FUM, ME, α- GP II, and F16Dh subunit structure are not understood because heteropolymers do not resolve into discrete bands on our gels. We were unable to resolve these latter 2 systems sufficiently to include them in the cage studies. Other alleles at α-GP II show dimeric patterns in a field population of *A. ervi* recently established at Escondido, California. No other polymorphic loci have been observed in any *A. ervi* stocks ; however, these laboratory cultures are several years old and most were not assayed as early in their laboratory regime or as thoroughly as the Japanese stock. Fixed allelic differences exist among some of the older stocks of *A. ervi* at GOT, α-GP II, ME, EST I, EST II, PGI and F16Dh.

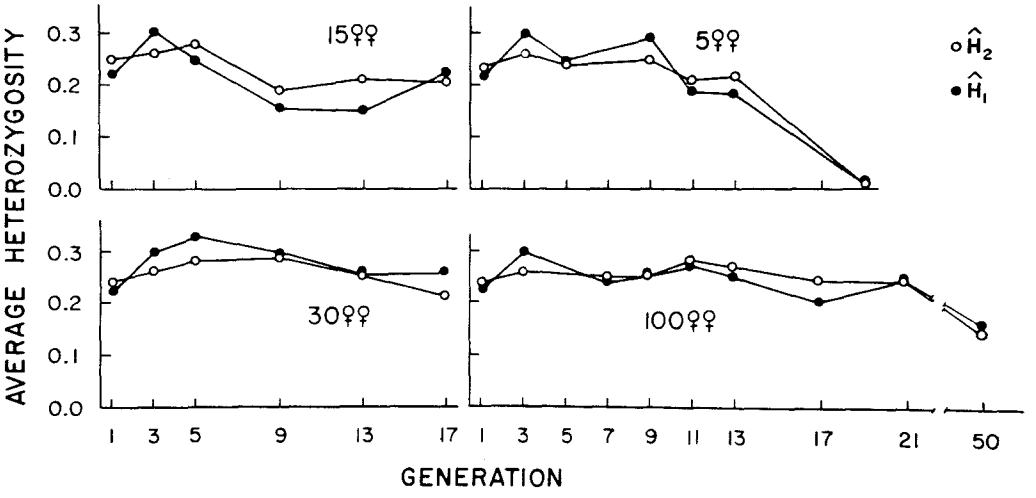

Fig. 3. Shown are the average heterozygosity values for several generations in each of 4 population cages maintained in the insectary. These 4 populations were renewed with 100, 30, 15, and 5 mated ♀♀ each generation. The values shown for the 1st and 3rd generations are identical for all 4 populations because they represent the ancestral stock that gave rise to these populations before it was subdivided (see fig. 1). $\hat{H}_1$ and $\hat{H}_2$ are the observed proportion of heterozygous individuals per locus and the expected proportion heterozygous individuals per locus derived from the Hardy-Weinburg principle (see text).

POPULATION CAGES

The pattern for the average heterozygosity ($\bar{H}$) in the 4 caged populations maintained under constant insectary conditions is depicted in figure 3. Two different measures of average heterozygosity were employed. The simplest, $\hat{H}_1$, represented by the solid circles in figure 3, is the average proportion of heterozygous individuals per locus, calculated directly from the phenotypes on the gels. The 2nd measure, $\hat{H}_2$, represented as open circles in figure 3, is the expected proportion of heterozygous individuals per locus ($= \sum_{i=1}^{n} 2p_i q_i/n$ where p_i and q_i are the allelic frequencies at the *ith* locus and n is the number of loci) derived using the Hardy-Weinburg principle. This statistic provides the proportion of heterozygous individuals expected when there is random mating and no selection, mutation or immigration. If siblings and other close relatives avoid mating with each other, then $\hat{H}_1$ will be larger than $\hat{H}_2$. Higher survival of heterozygous individuals produces a similar result. The converse processes will cause $\hat{H}_2$ to exceed $\hat{H}_1$.

There is an inconsistent relationship between $\hat{H}_1$ or $\hat{H}_2$ among population cages or within any given cage through time, suggesting that the observed differences arose by chance. A high level of inbreeding or outbreeding was not expected nor was it expressed. $\bar{H}$ increased from F_1 to F_3 (there was only 1 cage in F_3 ; see figure 1) and continued to increase or remain constant until F_9 in the 5 and 30 ♀♀ lines and until F_{11} in the 100 ♀ line. Heterozygosity in all lines tended to decline thereafter.

The actual allelic frequencies from which $\hat{H}_2$ was calculated are shown in figure 4. They show no remarkable trends. As expected, electromorph frequencies fluctuate more in cages maintained with fewer females.

If we assume random mating without selection the expected amount of heterozygosity maintained for a given nomber of generations can be calculated (**Li**, 1968) :

$$H_t = H_i\,(1 - (1/2N_e))^t \qquad (1)$$

Where H_t, H_i, and N_e are heterozygosity at time t, initial heterozygosity and effective population size, respectively. The effective population size is a theoretical estimate of the number of individuals which participate in the reproductive effort. The expected amount of heterozygosity retained, assuming the appropriate effective population size (for sex linked loci) is presented in table 2 with the observed amount of remaining heterozygosity estimated with both measures, $\hat{H}_1$ and $\hat{H}_2$, introduced earlier. The data include the High, Low and Outside 30 ♀♀ populations as well as the 4 insectary maintained populations. Effective population size is calculated with the following assumptions : 1) N ♀♀ are transferred each generation. 2) Each ♀ carries the sperm from a single mating. (Females rarely mate a 2nd time several days after an initial mating). 3) The sex ratio in the cages is 1 :1. (The sex ratio varied between cages and generations but tended to slightly favor ♀♀). The effective population size for M ♂♂ and F ♀♀ with a haplodiploid genetic system (♂♂ are haploid and ♀♀ are diploid) is identical to that for a sex linked locus : $N_e = 4.5MF/(F + 2M)$ (**Crozier**, 1976). With a 1 :1 sex ratio, N_e is 3/4 that of a diploid-diploid population. Because our ♀♀ were mated, they represented twice the number of individuals that were actually transferred or an effective population size 1.5 times the number transferred.

The 15 ♀♀ line lost heterozygosity more slowly than expected. The 30 ♀♀ line, reared under constant insectary conditions, lost heterozygosity at a rate very near to the predicted rate. The remaining 5 populations lost heterozygosity at a rate considerably higher than expected. The observed effective population sizes (see table 2) ranged from 19 % to 131 % of the predicted values with an average of 48 % of predicted. Thus, after correcting the predicted

effective population size for haplodiploidy, it is still twice as large as the observed effective size. This may have resulted from inbreeding behavior or selection but the most likely explanation is that all individuals did not participate equally in the reproductive effort.

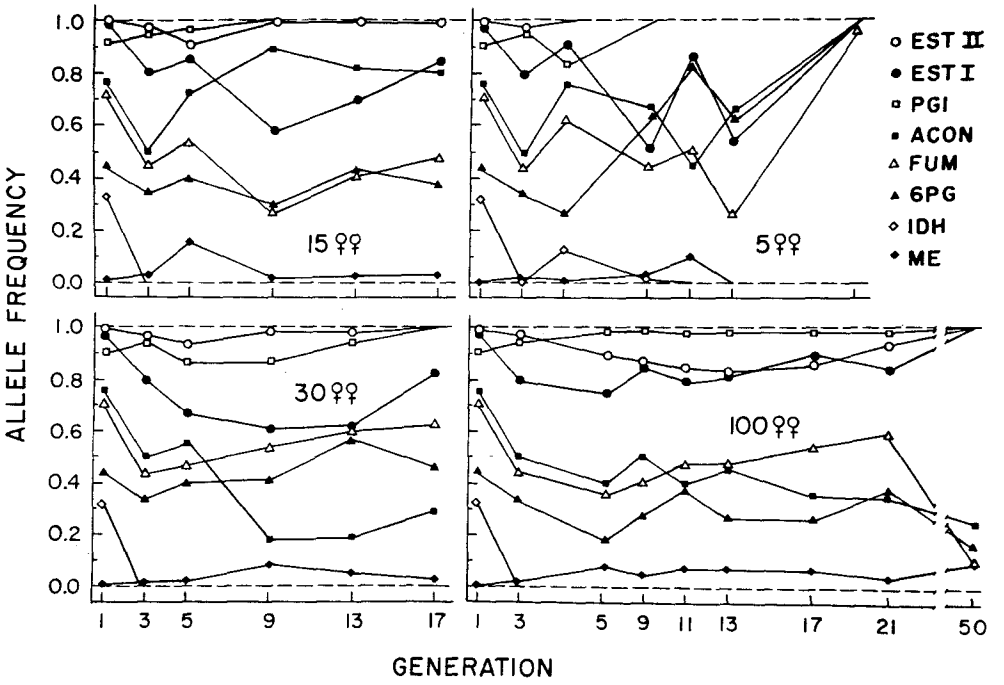

Fig. 4. Shown are the allele frequencies from which average expected heterozygosity ($\hat{H}_2$) in fig. 3 was calculated. When a line passes into either the upper or lower boundary (corresponding to allele frequencies of 0 or 1) then that allele has reached fixation. Each of the 8 loci depicted had a polymorphism which included only 2 allelic classes. Some heterogeneity existed within allelic classes but has been pooled for this study.

From the experiment using individually isolated ♀♀ for progeny production the following results were obtained. Of 144 ♀♀ utilized, 39 (= 25 %) produced no progeny, 5 (= 3 %) produced pure ♂ broods (all of which were a single individual per brood). The remaining ♀♀ produced 1 or more ♀ progeny demonstrating that they had been inseminated. The proportion of ♀♀ producing progeny was affected by the day they were aspirated from the cages. Grouping the 5 all ♂ broods with the zero broods we found that ♀♀ from the 1st, 2nd and 3rd days of emergence produced 14.5 %, 25.0 % and 41.7 % zero broods, respectively. Sex ratio in this test strongly favored ♀♀ (74.5 %), however, because mated ♀♀ were transferred in the population cage experiments, the effective sex ratio was near 1:1. This assumes that the ♀♀ that were transferred all had unique mates, a difficult assumption to test. The individual ♀ experiment demonstrates that uneven participation by ♀♀ in reproduction is an important factor in lowering effective population size. The test also justifies the assumption that ♀♀ were mated before they were transferred.

TABLE 2

Observed and expected values of the proportion of heterozygosity retained for the number of elapsed generations specified for each population cage. Also presented are the per generation loss rate of heterozygosity and the effective population size for both predicted values and observed data

			Expected			Observed I[a]			Observed II[b]		
# mated ♀♀ transferred/gen.	Temperature regime	Elapsed generation	Effective[c] population size	Proportion heterozygosity retained	Heterozygosity loss rate per generation	Effective population size	Proportion heterozygosity retained	Heterozygosity loss rate per generation	Effective population size	Proportion heterozygosity retained	Heterozygosity loss rate per generation
100	Insectary	47	150	.85	.0033	35.7	.52	.014	38.5	.54	.013
30	Insectary	14	45	.86	.011	50.0	.87	.010	31.3	.80	.016
15	Insectary	14	22.5	.73	.022	25.0	.74	.020	29.4	.79	.017
5	Insectary	16	7.5	.33	.067	2.0	.01	.250	2.0	.01	.250
30	High	5	22.5	.95	.011	10.9	.79	.046	8.6	.74	.058
30	Low	8	22.5	.91	.011	21.7	.83	.023	12.5	.72	.040
30	Outside	6	22.5	.94	.011	10.6	.75	.047	14.3	.81	.035

[a] Calculated from the average proportion (per locus per individual) of observed heterozygotes.

[b] Calculated from the average proportion (per locus per individual) of expected heterozygotes based on the allele frequencies using Hardy-Weinburg principle ($H = 2pq$; see text).

[c] Based on the assumption that males are haploid and females are diploid ; effective size = 3/4 that of diploid-diploid population when sex ratio = 1.

DISCUSSION

The genetic problems inherent in biological control programs have recently been reviewed (**Mackauer**, 1972, 1976). In this study we have considered genetic drift arising from sampling populations in laboratory culture. Unfortunately, most, if not all, phases of a biological control project represent potential genetic bottlenecks including : sampling the population in its native home, importation, quarantine, subsequent laboratory culture, and even release and establishment (*contra* **Mackauer**, 1976).

The genetic drift which we have documented in *A. ervi* laboratory cultures is functionally no different than genetic drift at any other phase of a biological control program. With each generation, the population is sampled and so, too, is its array of genetic variability. Significant differences exist between each phase in their inherent propensity for genetic bottlenecks (the number of individuals sampled) and the kind and intensity of selection that may occur. These attributes will also differ among different natural enemy species because of their genetic population structure, breeding system, ease of collection and rearing, etc. Despite this diversity a simple generalizable conclusion can be drawn from our results and those of others. Genetic drift in laboratory culture (and other phases of a biological control program) is more extreme than the number of individuals actually sampled would indicate. This, in itself, is an important warning to biological control workers.

The 30 ♀ cages (30, High, Low and Outside), which reflect the typical methods previously employed for aphidiines at Riverside, displayed an average rate of decline in $\hat{H}_2$ per generation of 0.03725. This is about 4 times the expected rate and reflects an effective population size of only 18 individuals (or 9 mated ♀♀) compared to the expected N_e of 45 (22.5 mated ♀♀) when haplodiploidy is considered. At the observed rate heterozygosity would be halved in 18 generations or in less than 9 months in culture. This is not an unusually long time for a culture to be maintained prior to release.

Effective population size is an important concept in understanding why heterozygosity decreases rapidly in small laboratory colonies. Effective population size is a weighted average of the number and degree to which individuals participate in the reproductive effort. Based on empirical evidence we believe that insect cultures, in general, are being maintained with effective population sizes much smaller than apparent population sizes. **Kerr & Wright** (1954) found that the effective population size was 83 % of the number of individuals sampled each generation in a study of a neutral polymorphism in 96 cages of *Drosophila melanogaster* Meigen. When a polymorphism included a deleterious allele, selection made effective population size appear even smaller. When a heterotic combination was studied, effective population size appeared larger than the actual number of individuals transferred each generation. Their study points out how selection and genetic drift can combine to reduce genetic variability and how, in some cases, drift and selection can oppose each other. In another study **Prout** (1954) found that effective population size was actually 1/4 of the 1000 individuals that were sampled each generation. Clearly, even in diploid insects effective population size can be much smaller than the actual population size.

One force that will reduce effective population size more than is intuitively obvious is fluctuation in population size. Effective size for a sequence of generations is their harmonic mean. For example, a population with sizes of 100, 10 and 100 individuals in 3 successive generations will have an effective population size of only 25 individuals, other things being equal. This "geometric effect" of population bottlenecks may account for some of the unexplained loss of variation in the *A. ervi* cages. Other forces which will reduce the effective population size

include haplodiploidy or sex linkage as has already been discussed, high variation in parental contribution to the next generation and highly skewed sex ratios (**Spiess**, 1977).

Biological control workers know that genetic drift should be avoided by maintaining large population sizes. Other principles concerning genetic drift are less generally known. Given that a population bottleneck does occur, maximizing population growth after the bottleneck will strongly reduce its effects on average heterozygosity but not on allele loss (**Nei** *et al.*, 1975). Furthermore, as this study indicates, effective population size may be far smaller than their apparent size. We surmise that the only way to prevent genetic drift is to keep population sizes large. However, an alternative method has been suggested which is based on the stepping stone model of population structure (**Wright**, 1951).

When a population is subdivided into several smaller subpopulations (= stepping stones) among which gene flow may occur, the decline of heterozygosity can be less than that predicted by equation 1, given assumptions about the size and number of subpopulations and the amount of gene flow among them. The circular stepping stone model analyzed by **Muruyama** (1970) consisted of a circle of n colonies each having N breeding individuals and exchanging individuals with adjacent colonies at a rate of m per generation. He found that when $m < n/\pi^2 N$, heterozygosity decays at the rate $m\pi^2/2n^2$, which is independent of population size, proportional to the migration rate and inversely related to the number of "stepping stones". However, examination of this protocol indicates that its effect hardly differs from that obtained by producing several inbred or isofemale strains (initiated from the progeny of a single mated ♀). That is, with 5 (n) subpopulations of size 30 (N) the migration must be less than one individual per 60 generations for heterozygosity to decay at the reduced rate, $m\pi^2/2n^2$. If migration occurs more often, then heterozygosity decreases at the rate $1/2nN$ (= eqn 1). Clearly, the only outstanding alternative to large culture sizes for maintaining genetic diversity is many inbred lines. Whether variability maintained in stocks of inbred lines can be reconstituted into some semblance of the structure of the originally sampled population remains an open question (**Wright**, 1980).

We believe that inbred lines do not presently represent a practical alternative for maintaining genetic variability in biological control programs. Inbred lines are a research technique to be used, for example, when maintenance of a specific genetic trait is required. Unfortunately, much of the theory upon which the above conclusions rest evolve from assumptions that ignore epistatic interactions, chromosomal linkage of traits and other important biological realities that may be important in explaining the adaptive diversity of organic life (**Lewontin**, 1974).

Finally, what of genetic variation that may be selectively important but is not easily assayed, including such characters as diapause, temperature tolerance, and behavior ? Variation exists among populations and often may reflect local adaption. Selective forces, interacting with inbreeding and drift can reduce field adaptedness and vigor when insect populations are cultured for more than a few generations. Laboratory strains of *Trichogramma chilonis* Ishii (= *T. confusum*) displayed higher ♀ sterility, less temperature tolerance and lower progeny production than did wild strains (**Nagarkatti & Nagaraja**, 1978 ; **Nagarkatti**, 1979). A wild strain of *Anastrepha suspensa* Loew, on the other hand, laid fewer eggs, had lower proportions of eggs hatch and a longer generation time than an old laboratory culture. The wild strain laid more eggs in culture each succeeding generation for 5 generations, suggesting adaption to the laboratory was taking place (**Leppla** *et al.*, 1976). But what was happening to this strain's field adaptedness ? Clearly, variation in adaptive traits exists in natural populations. It is this variation in adaptive traits that we seek to identify and preserve in biological control (**Gonzalez** *et al.*, 1979). Protein polymorphism accounts for a small percentage of the adaptive potential of populations, thus direct and easy correlations are to be discarded. But protein polymorphisms provide useful

information on genetic drift in small populations, a process which may act on adaptive traits as well.

Unique electrophoretic loci may exist in many populations of a species which can be used as markers to identify a specific trait or geographic origin. Successfully established populations can be compared to the originally imported material for heterozygosity, specific allelic frequencies, sex ratio and more basic information such as linkage disequilibrium. Indeed, if we can identify geographic origin(s) of established parasite populations introduced from several regions in the world, then we have the foundations for a less empirical framework for foreign exploration and associated efforts in biological control.

Because classical biological control requires the movement of populations into new and different environments we must develop an understanding of the adaptive diversity populations represent. When we move populations, we create experiments, perhaps crude, which can shed some light on these questions. Genetic drift, as well as inbreeding and selection occurring in founder colonies, transport, quarantine and culture of natural enemies, will deter us from reaching our goals until we grasp the nature of variation within and among populations.

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RÉSUMÉ

Hétérozygotie et importance des populations de laboratoire de *Aphidius ervi* [*Hym. : Aphidiidae*]

La diminution de la variabilité des allozymes dans 7 populations de laboratoire de *Aphidius ervi* Haliday montre que l'importance des effectifs des populations correspond approximativement à la moitié du nombre d'individus utilisés pour renouveler l'élevage à chaque génération. Une participation inégale à la reproduction des individus femelles aux générations successives est la cause la plus probable de ce phénomène.

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