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Linda Jean Mason
Dept. of Entomology
Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Baton Rouge, LA

Moderator & Editor

THE LABORATORY AS AN ALTERED HABITAT: PHENOTYPIC AND GENETIC CONSEQUENCES OF COLONIZATION

L. J. MASON, D. P. PASHLEY[1], AND S. J. JOHNSON
Department of Entomology
Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center
Baton Rouge, Louisiana 70803
and
[1]Department of Entomology
Louisiana State University

ABSTRACT

Colonization of insects can cause dramatic changes in both phenotypes and genotypes. Phenotypic changes have been reported to occur in host plant acceptability, pheromonal responses, and reproductive parameters. Genetic alterations can include loss of genes, decreases in heterozygosity, and shifts in allele frequencies. The latter may be caused by random genetic drift, founder effects, nonrandom mating, and selection. Monitoring of laboratory quality can be accomplished by extensive record keeping on performance but a more efficient way is starch gel electrophoresis. Examples of this method applied to a soybean looper (*Pseudoplusia includens)* (Walker) laboratory colony and several fall armyworm (*Spodoptera frugiperda* (J. E. Smith)) populations are presented. Soybean looper heterozygosity decreased from 0.216 to 0.083 after two years of colonization. Fall armyworm colonies exhibited more variable heterozygosity levels and, in general, genetic distances were higher among colonies than field populations.

RESUMEN

La colonización de insectos pueden causar cambios dramáticos tanto en los fenotipos como en los genotipos. Se han reportado cambios que han ocurrido en la aceptabilidad de plantas hospederas, reacción de feromonas y parámetros de reproducción Alteraciones genéticas pueden incluir pérdidas de genes, disminución de heteregosidad, y cambios en la frecuencia de aleles. Lo último puede ser causado por la deriva genética al azar, efectos de fundación y del apareamiento y selección no al azar. Se puede realizar un chequeo de la calidad del laboratorio manteniendo un récord extensivo del comportamiento, pero un ;medio más efectivo es el de electroforesis de gelatina de almidón. Se presentan muestras de este método aplicado a una colonia de laboratorio del medidor de la soya (*Pseudoplusia includens*) Walker y a varias poblaciones de cogolleros (*Spodopetera frugiperda*) (J. E. Smith). La heterogosidad del medidor de la soya disminuyó de 0.216 a 0.83 después de dos años de colonización. Colonias de cogolleros exhibieron niveles de heterogosidad más variable y en general, las distancias genéticas fueron mayor entre colonias que en poblaciones de campo.

Insects have been cultured since at least 4700 BC (Hoy 1976) when the Chinese began rearing silkworms, *Bombyx mori* (L.), for sericulture. Over the centuries, the silkworm trade has emphasized that "every 'educator' of silkworms should be keenly alert to note and care for such silkworms as are more robust and forward, and by giving them attention, obtain better eggs for broods, and so secure constant improvement in the industry" (Minns 1929). More directed strain improvement began in the 1700's with rigorous artificial selection and continued into the 1800's by exchanges of strains among villages for hybrid production. Today more sophisticated genetic approaches are used such as controlled breeding for advantageous characteristics and crossing of different varieties to increase vigor.

Insect colonies are now maintained to supply research insects for many purposes including insecticide resistance studies, behavioral and physiological investigations, and as food sources for other insect colonies. Colony sizes can vary from millions when individuals are to be used in sterile release programs to only a few, as in invertebrate zoos. For most purposes, it is important to maintain insects that are of high quality and closely resemble wild organisms. However, this is not always realized. During the first few generations after colonization, laboratory productivity often decreases, then as adaptations to laboratory conditions increase, productivity is enhanced (Hoy 1976, Zouros et al. 1982). Laboratory quality usually is defined in terms of how well an insect population functions in its intended role either in the field or laboratory (Huettel 1976, Bush 1979). It is ironic that large amounts of time and money are often spent on experiments utilizing laboratory insects, but little is spent on maintaining their quality (Roberson 1984).

PHENOTYPIC CHANGES DURING COLONIZATION

The underlying cause for phenotypic differences observed between laboratory and field populations can be either environment induction or genetic differentiation. Environmental factors such as temperature and diet may induce changes in oviposition rates or color, respectively. If these are undesirable traits, removal of the environmental stimulus may correct such problems.

Genetic changes can have much more permanent and profound effects. For example, a change in ovipositional rate due to increased temperature will return to normal at low temperature, but genes that are lost due to sampling error or selection do not return. When this occurs it is difficult to correct without enriching the gene pool from external sources (e.g., natural populations).

Numerous cases have documented phenotypic changes during colonization. Specific examples include host plant acceptability, pheromone response, visual sensitivity, flight capacity, and many reproductive parameters such as mate choice, fertility, ovipositional patterns, and mating frequency. Schoonhoven (1967) showed that laboratory-reared *Manduca sexta* (L.) accepted host plants that wild individuals rejected. He determined that a change in the sensitivity of contact chemoreceptors had occurred in laboratory-reared tobacco hornworms which allowed them to more readily detect acceptable chemicals in plants foreign to them. Thus, increased sensitivity to acceptable chemicals resulted in a shift in food preference.

A change in pheromone response was observed in the screwworm fly (*Cochliomyia hominivorax* (Coquerel)) colonies from Mexico and Florida that had been reared under different conditions (Fletcher et al. 1968). Mexican colony females did not respond to pheromones of their own strain or the Florida strain, while the Florida strain responded to both. By rearing the Florida strain in constant darkness, selection pressures may have preferentially favored utilization of odor for mate location. The Mexican strain had recently been reared in light.

In a comparsion of visual sensitivity of wild and laboratory screwworm flies, Goodenough et al. (1977) examined differences between wild, mass-reared nonreleased, and mass-reared recaptured flies. Wild and mass-reared recaptured females has a greater visual sensitivity than mass-reared nonreleased females. They concluded either a change in rearing techniques or replacement of the laboratory strain was in order to maintain wild behaviors in the laboratory.

Flight capacity is a behavior that can be affected by changes in diet that often accompany colonization. Olive fruit fly females (*Dacus oleae* (Gmelin)) reared in the laboratory on artifical diet had a 58% reduction in flight capacity and males had a 67% reduction when compared with those reared on olives or with wild flies (Remund et al. 1977). It was suggested that rearing the flies in small cages with food, water, and ovipositional surfaces within close proximity selected for shorter flight capacities. A similar observation was reported by Fletcher and Economopoulos (1976) who found that laboratory-reared olive fruit flies dispersed over much shorter distances than their wild counterparts.

Reproductive behavior is greatly affected by colonization and changes not only affect quantitative aspects of production but also quality. Male *Anopheles quadrimaculatus* Say raised and sterilized in the laboratory were able to find and mate with released colony females but were unable to find wild females (Dame et al. 1964). Therefore, the goal of the release program, a decrease in wild female fertility, was not achieved.

Using one wild strain and two laboratory strains of *Musca domestica* (L.), Fye and LaBrecque (1966) demonstrated that laboratory females preferentially select males of their own strain rather than wild type males when given a choice. Additionally, when females of a wild strain were bred to wild strain males, their fertility was lower than a similiar cross between laboratory individuals.

A laboratory strain of tobacco budworm, *Heliothis virescens* (Fabricius), that was about 90 generations old was found to have a greater percentage and frequency of mating when compared with wild strain individuals (Raulston 1975). Ovipositional patterns also significantly differed in that the laboratory strain oviposited earlier than the wild strain. However, after the wild strain had been reared in the laboratory for three generations, it was not significantly different from the other laboratory strain. Thus, in only three generations of laboratory selection, wild characteristics were eliminated.

Along these same lines, Economopoulos et al. (1976) observed that females reared on an artificial diet in the laboratory oviposited three to four times more during the first four to five weeks than those fed on olives. In other words, feeding on artifical diet resulted in high rates of reproduction early in life. This could also occur if oviposi-

tional containers were only maintained for a short period of time and late reproducers were unable to oviposit. Six years later it was reported that during the first mating period, laboratory flies mated earlier in the photoperiod than wild flies (Zervas and Economopoulos 1982). It was suggested that this was possibly due to adaptation to the laboratory light:dark regime and not dietary influences.

One of the first discoveries linking a phenotypic change to a genetic change came with the development of the screwworm eradication program. The mass release of sterilized flies from 1962 to 1971 successfully suppressed wild populations of screwworms in the southwestern United States. However, in 1972 the number of reported cases increased approximately 200-fold (LaChance 1979). Although it decreased somewhat in the following four years, it never returned to the pre-1972 levels (Bush and Neck 1976). Bush et al. (1976) were the first to examine the loss of competitive ability of the mass-reared flies using electrophoresis. They determined that mass-reared insects were fixed for an allele of alpha-glycerol phosphate dehydrogenase that was rare in the wild flies. This enzyme is involved in the glycerol phosphate shuttle and its activity was demonstrated to be temperature sensitive (Zebe and McShan 1957). Energy generated through this metabolic pathway is crucial to strong flight (Kitto and Briggs 1962). Laboratory colonies contained weak fliers which were not able to disperse and find mates in the wild. Rearing conditions such as extensive surface area for walking, total darkness, and high constant temperature, presumably favored noncompetitive flies. They suggested that by altering conditions in which the flies were raised, quality would be improved and that electrophoretic monitoring could serve as a check for at least one important fitness character. When the old colony was replaced with a new colony lacking the defective allele, reported cases again dropped to the pre-1972 level (Bush 1979).

Another connection between phenotype and genotype was made by Zouros et al. (1982). Laboratory-reared olive fruit flies had a 40-fold increase in the frequency of a rare allele at the alcohol dehydrogenase (ADH) locus within six generations. The frequency of the most common alleles in turn decreased more than 50% over the same time period. The ADH locus may be associated with larval viability on diets with various levels of alcohols. It is noteworthy that major genetic changes occurred within the first two generations. The fact that changes in genetic composition can occur rapidly might explain why Economopoulos (1972) found no differences in mating preference between an established colony of laboratory flies reared on artificial diet and third-fourth generation laboratory flies reared on olives.

In another genetic study, Berlocher and Friedman (1981) found that a 10-15 year old laboratory colony of the black blowfly, *Phormia regina* (Meigen), had considerably less genetic variation than wild flies or first-generation laboratory colonies. Heterozygosities decreased from 0.157 in the wild to 0.009 in one of the laboratory colonies. Pashley and Proverbs (1981) found that during the process of codling moth (*Cydia pomonella* (L.)) colonization, there was no significant decrease in heterozygosity over 25 generations, but there was significant allozyme frequency change at three out of five loci examined. Although ascribing a specific cause for an observed change in genotype or phenotype is difficult, there are certain factors that have characteristic effects and those are outlined in the next section.

Causal Factors Associated with Genetic Change

Causes of genetic change include random genetic drift, founder effect, nonrandom mating and selection. Each process can have a characteristic effect on allele frequency changes, but in some cases the net result may be the same. Additionally, the processes may either act individually or in concert, complicating their resolution.

In small populations, such as those in many laboratory colonies, sampling error may cause gene frequencies to fluctuate between generations. The smaller the population, the greater the fluctuation. These fluctuations can cause fixation or loss of alleles from the population in a short period of time and may alter gene frequencies.

Founder effects can occur if the initial laboratory colony is small in comparison with the field population or when the colony size is reduced such that a "bottleneck" occurs. Only a small amount of genetic variation present in the field population may be contained in the laboratory colony because average heterozygosity and the mean number of alleles per locus decreases. Nei et al. (1975) showed that the reduction in average heterozygosity per locus is due to the size of the bottleneck and population growth after the bottleneck. However, the number of alleles present is entirely dependent on bottleneck size (i.e., once lost, alleles are not recovered) and rare alleles (i.e., low frequency alleles) have a higher probability of loss than common alleles. Losses that are a result of founder effects and bottlenecks may be restorable with proper laboratory management through introduction of wild insects.

Nonrandom mating such as inbreeding can also cause a reduction in average heterozygosity while increasing homozygous classes. This may or may not lead to changes in gene frequency. If genes are exposed to natural selection while in the homozygous condition, gene frequencies may change. If positive assortative mating occurs, homozygosity will also increase but only at the locus or loci that are involved in mate choice or those that are linked to them. Other loci should remain in Hardy-Weinberg equilibrium. When inbreeding is extreme, "inbreeding depression" or a reduction in fitness may occur. This loss of fitness may be manifested by reduced size, fertility, or vigor, all of which affect laboratory quality.

Selection in nature is a generally accepted phenomenon but it can occur just as readily in the laboratory, either intentionally or inadvertantly. Initial selection can occur by simply bringing field populations into the laboratory and rearing them at constant temperatures on an artificial diet. Genotypes that do not readily adapt to the laboratory environment may be eliminated. If selection is affecting only one locus, changes may occur at that locus and not at others. This may be obvious when examining the results of an electrophoretic survey on the colony. If enzyme function can be correlated to a specific aspect of the rearing procedure, alternative rearing procedures may prevent allelic changes.

A common thread in the above processes is a change in average heterozygosity which generally reduces genetic variability. The importance of this reduction has been investigated thoroughly (see Hedrick et al. 1976 for a review) and while the mechanism may vary, the relationship of genetic polymorphisms to the general well being of a colony is strong. Because the laboratory environment is usually quite different from the field, it is not surprising that genetic changes in the laboratory can result in lowered fitness and competitive ability.

Monitoring Genetic Composition

The most obvious way to detect change in a laboratory colony is by careful monitoring for phenotypic changes in such areas as flight capacity, mating propensity, ovipositional rate, survivorship, etc. Keeping a daily record of a colony's performance will give early indications that changes are occurring. One major drawback, in addition to the time investment, is that large genetic changes may have already occurred when phenotypic changes are obvious. A more expedient method for early detection of genetic changes is starch gel electrophoresis. Through this procedure one can readily determine changes in genetic structure by the examination of protein products of the DNA that have been repeatedly demonstrated to have a genetic basis.

In an electrophoretic survey of six enzyme loci, field-collected soybean loopers, *Pseudoplusia includens* (Walker), and two laboratory colonies were compared. An analysis of heterozygosities at generation four (Lab 1) and approximately two years later (Lab 2) indicated a decrease over time (Fig. 1). Causes for the decrease are only speculative but suggest a compromise in quality. Five colonies of fall armyworm, *Spodoptera frugiperda* (J. E. Smith), were analyzed and compared with two field populations collected on three different host plants. Two host strains, one from rice and Bermuda grass and the second from corn, exhibited significant genetic differentiation (Pashley et al. 1985, Pashley 1986). Georgia, Alabama and Louisiana (3) colonies had heterozygosity values much lower than field populations (Fig. 2; see caption for collection sites, hosts and year). The Georgia colony exhibited extreme effects of colonization. The Alabama colony has not had new material introduced since its initiation approximately four years ago. Colonization does not necessarily reduce heterozygosity values as shown by the Mississippi and Louisiana (2) colonies. In the latter case the value was even higher than the field population. This increase may be due to sampling effect and subsequent frequency shifts which increase heterozygosity. It serves to illustrate that loss of heterozygosity is not a required phenomenon.

In a multilocus summary of genetic relationships of fall armyworm, Louisiana (2) was similar to the rice/Bermuda grass strain while the other four colonies were more similar to the corn strain (Fig. 3). These results are consistent with the original host sources for the colonies. If more than one host strain is pooled to form a colony, it may appear intermediate between strains. This may explain the high genetic distance between the Alabama colony and the two field strains.

Within a given strain, genetic distances between field collected populations and lab colonies generally exceed that among natural populations. In a survey of 23 samples of the corn strain from Central America, the Caribbean, and the southeastern U.S. (Pashley 1986), the maximum Nei's distance (in a phenogram) was slightly below that between the corn strain and Mississippi (Fig. 3.). Thus, with the possible exception of Mississippi, colonies are more divergent from their natural progenitors than any of the natural populations are from one another, even across very large geographic distances (e.g., Costa Rica and Florida). Divergence at these allozyme loci is presumably indicative of changes in other genes. If so, the colonization process results in a genetic composition quite different from that found in wild individuals.

We have summmarized the importance of monitoring genetic composition of laboratory-reared insects. Much of the work published so far has been directed toward the release of a laboratory-produced insect into the field which can compete with wild individuals. This may not be the goal of those who use insects as research tools in such areas as toxicology and host plant resistance. However, if research results are to be used to generalize about activity or responses of field populations then high priority must be given to maintaining laboratory quality. In short, laboratory individuals must be genetically similar to their field progenitors.

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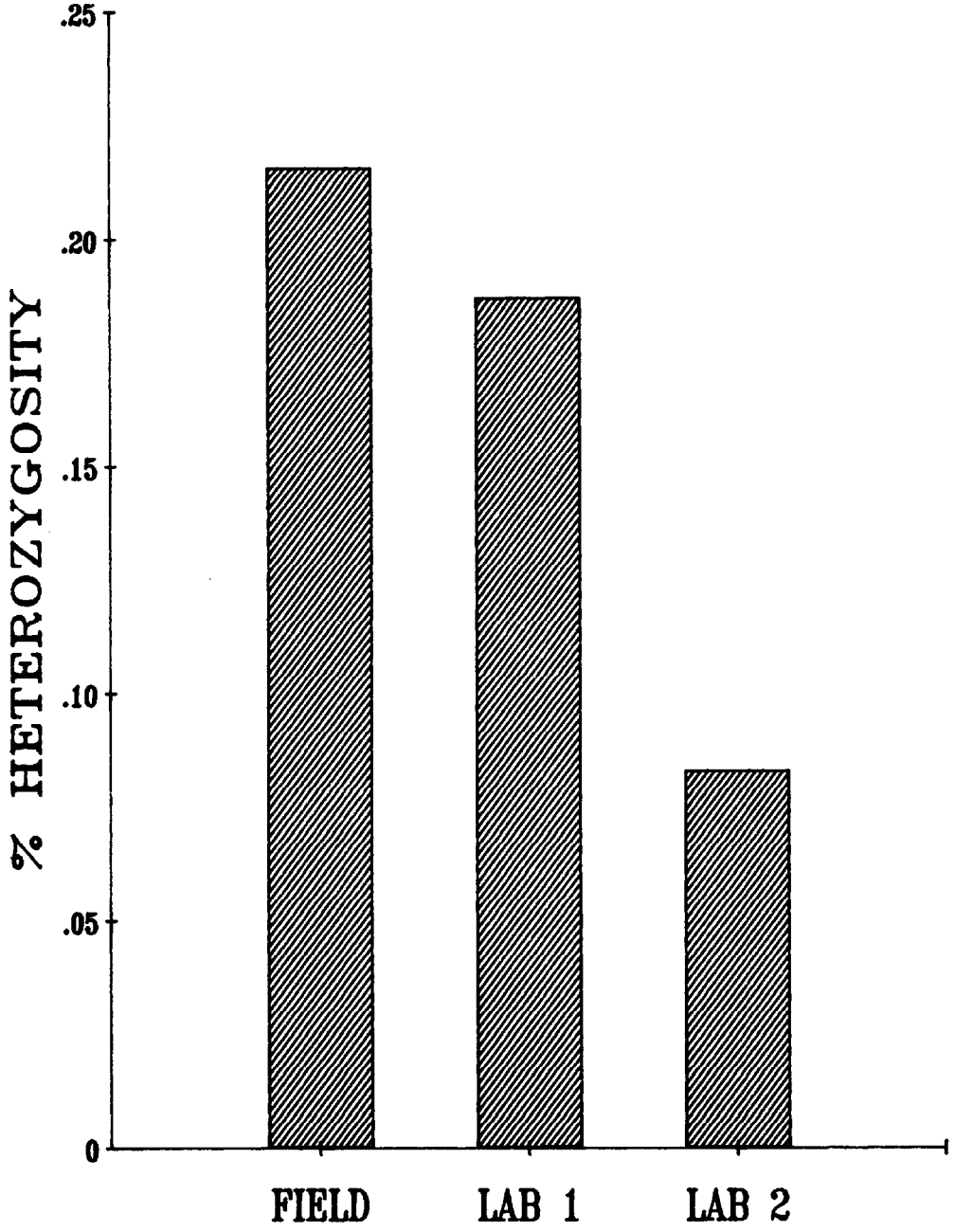

Fig. 1. Heterozygosity values of soybean loopers from three sources. Field = wild population collected on soybeans in St. Gabriel, LA. in 1983 (N (no. of individuals analyzed electrophoretically) = 32); LAB 1 = 4th generation laboratory colony established from the above Field population (N=54); LAB 2 = 24th generation laboratory colony established from the Field population (N=61). Values based on six polymorphic enzyme loci.

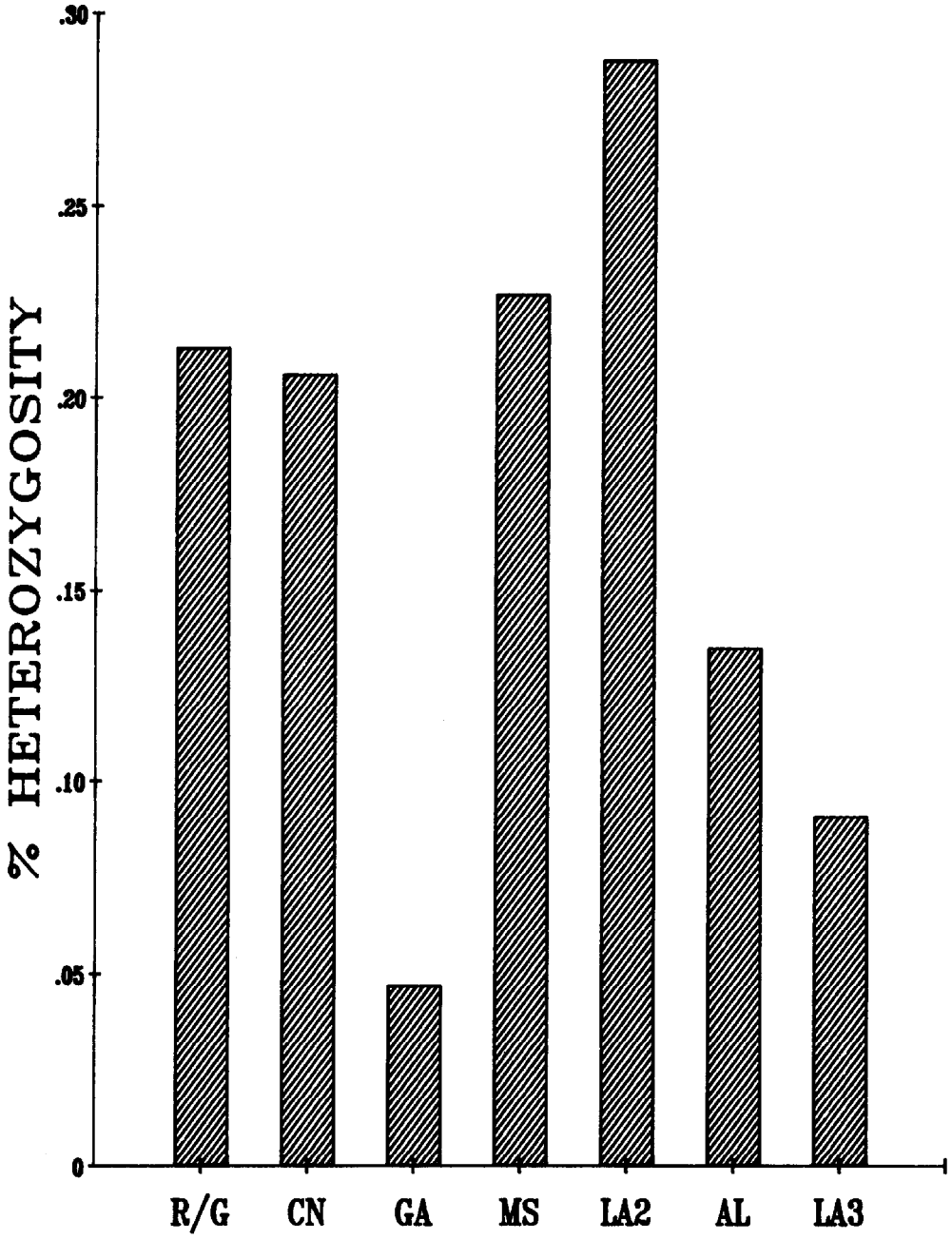

Fig. 2. Heterozygosity values of fall armyworm from field collections on three host plants. R/G = rice from Puerto Rico and Bermuda grass from Baton Rouge, LA., 1985 (populations were identical and thus pooled), (N=121); CN = corn, Baton Rouge, LA. 1984-85, (N=136)) and several laboratory colonies; GA = Georgia USDA, corn, (N=32); MS = Stoneville, MS., 1984, corn (N = 33); LA2 = Louisiana, 1984, Bermuda grass (N=33); Al = Alabama, 1981, corn and grass (N=59); and LA3 = Louisiana, 1983, corn (N=20). Values based on seven polymorphic enzyme loci.

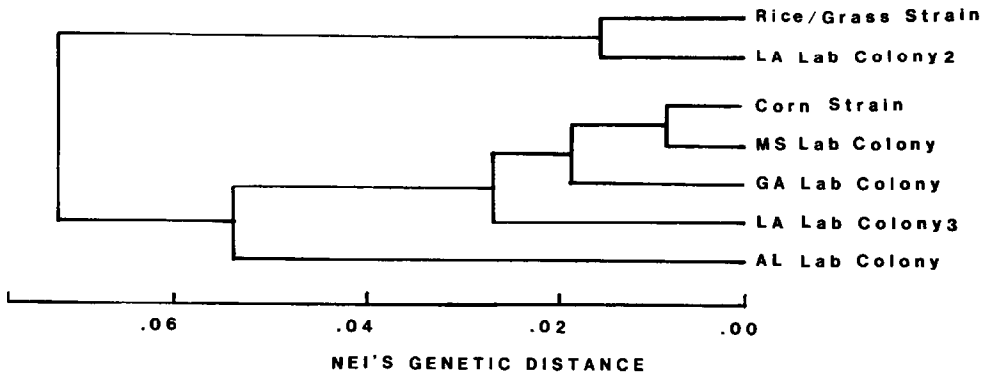

Fig. 3. Phenogram using an unweighted pair group method with arithmetric averaging (UPGMA) of genetic relationships based on seven polymorphic enzyme loci among fall armyworm colonies and field collections (see Fig. 2 for details concerning colonies). Cophenetic correlation coefficient = 0.96. Rogers' distances (1972) produced an identical pattern of UPGMA clustering.

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