Ecological Genetics of the Screwworm Fly, *Cochliomyia hominivorax* (Diptera: Calliphoridae) and Its Bearing on the Quality Control of Mass-reared Insects

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ABSTRACT

Electrophoretic variants at five loci have been studied in laboratory and factory (mass-production) adult screwworm flies. The pattern of genetic changes observed in several factory strains is very similar, but these changes differ from those which occur in smaller laboratory populations. Genetic variation in all colonies, however, decreases with colonization time. The most dramatic change occurred at the α-glycerophosphate dehydrogenase locus. This enzyme serves a key role in energy flow during flight and its activity is sensitive to temperature changes. An allele which is rare in natural Texas populations consistently becomes fixed, or nearly so, in each strain reared under factory conditions. These genetic changes are due to conditions peculiar to the factory, i.e., rearing at constant high temperatures, selection for rapid development time and reduced flight activity. The use of electrophoresis to measure genetic changes in ecologically important proteins as a quality control monitoring program is proposed.

From 1962–1971 the Screwworm Eradication Program conducted by the U.S. Department of Agriculture Animal and Plant Health Inspection Service (APHIS) managed to protect the Southwest from screwworm. This suppression campaign provided substantial savings to livestock producers and consumers. However infestations jumped from about 473 in 1971 to 94,551 during 1972 (Anon. 1973, Newton and Ferguson 1974). Although fewer cases were noted in subsequent years (1973, 9,000; 1974, 7,272; 1975, 17,568), infestations have never returned to the pre-1972 levels for reasons not yet fully understood (Bushland 1974).

Calman (1973) and Smith (1973) suggested that factory-reared flies and wild flies might be evolving simultaneously in different directions. Intense selection pressures generated by the release of over 10 billion irradiated adult *Cochliomyia* a year may have resulted in the rapid evolution of a wild strain that no longer mates readily with released flies on the one hand, while factory rearing conditions may have favored the selection of a domesticated strain that is no longer adapted to natural environmental conditions resulting in reduced competition with wild mates. Historical and economic aspects of the project have been reviewed by Baumhover (1966), Bushland (1974, 1975) and Steelman (1976).

Regrettably, no reliable methods for testing the mating propensity of factory reared flies under natural conditions have been developed. Mating between wild flies has not been observed under natural conditions, and information on the ecological factors and behavioral cues essential for normal mating are lacking. It has therefore been difficult to establish exactly why the wild flies can no longer be suppressed.

One important aspect of the fly's biology related to mating activities is the repair of the great flight capacity. Adults have been captured up to 180 mi from a release point and many flies travel at least 10 mi during their adult life (Hightower et al. 1965). Because natural population densities are normally low (>100–200 individuals/m2) (Bushland 1975, Lindquist 1955), flies must be able to disperse to find mates and suitable environmental conditions for survival and oviposition. To remain competitive with wild flies, factory reared flies must be able to disperse and mate under conditions encountered in nature.

At the time of the 1972 outbreak mass rearing methods were designed to discourage flight activity in order to reduce damage to the parental flies and increase egg production. Approximately 70,000 adults of the OLD and NEW APHIS strains were held in large cages at 75°C (+1°C) in total darkness. Each cage was subdivided by many large sheets of newsprint suspended from the cage ceiling to increase the surface area and to further inhibit movement. Food and oviposition sites were placed on the floor of the cage. A premium was therefore placed on the fly that walked rather than flew, or flew only short distances. Also, flies reared at a relatively high constant temperature were not exposed to the highly variable weather conditions individuals would encounter in nature. Selection was therefore relaxed for maintaining adequate flight muscle activity to insure normal dispersal in nature.

Enzymes involved in the direct or indirect regulation of energy flow through metabolic pathways appear to be particularly sensitive to selection (Johnson 1974). Therefore, we examined the effects of the factory environment on 2 polymorphic enzymes involved in flight activity. Gel electrophoresis was used to study the patterns of genetic changes occurring during colonization in α-glycerophosphate dehydrogenase (α-GDH) and phosphoglucomutase (PGM), regarded as Group I enzymes following Gillespie and Kojima (1968).

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In strong flying insects, α-glycerophosphate dehydrogenase (α-GDH), a key enzyme, governs the transfer of reducing equivalents from cytoplasmic NADH to the mitochondrial electron-transport chain by way of glyceraldehyde 3-phosphate (Zebe and McShan 1957, Zebe et al. 1959, Sacktor and Dick 1962). The amount of α-GDH present in flight muscle is strongly positively correlated with flying ability (Kitts and Briggs 1962, Brosemer 1967). Mutants of Drosophila melanogaster which lack α-GDH cannot fly (O’Brien and MacIntire 1972).

By controlling the concentrations of nucleotide co-factors, α-GDH may thus indirectly regulate metabolic rates (Johnson 1974). Phosphoglucomutase (PGM) is also an important regulatory enzyme on flight metabolism acting directly on the reaction glucose-G-P to glucose 1-P during gluconeogenesis.

Three variable substrate polymorphic enzymes (Group II) not directly involved with flight metabolism were also analyzed to see if mass rearing would lead to a similar or different pattern of change. These include leucine amino peptidase (LAP) which hydrolyses proteins and two esterases (EST-A, EST-B) which act in the hydrolysis of ester linkages.

Materials and Methods

Samples

Samples were taken from various strains over a one-year period. The OLD APHIS strain which was not able to control the outbreak in 1972 originated from larvae collected at several sites in northern Mexico in 1971. The NEW APHIS factory production flies being reared when the study was initiated and used in the 1973 eradication campaign originated from several thousand wound-reared larvae collected in Webb and Hidalgo Counties, Texas. The PUERTO RICO strain was developed from 25 egg masses collected in March 1972, from wounded animals on the island of Vieques off the coast of Puerto Rico. All 3 factory strains were reared under continuous light as larvae and in total darkness as adults to inhibit flight activity. A dim light was provided only at the time of oviposition.

The TEX-MEX strain then under development was synthesized by outcrossing adults reared from 6 egg masses collected on wounded animals in 3 northern Mexican states (MEX). Equal numbers of this population and a population derived from outcrossing the progeny of 18 egg masses collected on wounded cattle from 12 southern Texas counties (TEX) were then hybridized. The TEX-MEX strain adults were held until sexually mature (8 days) under diurnal light conditions (11 h 52 ft-c light with 1 h 0–52 ft-c dawn and dusk periods/24 h) under less crowded conditions (35,000–40,000).

Samples were removed at random from the TEX and MEX populations in May just prior to the time the 2 strains were combined to form TEX-MEX. Both the OLD APHIS and TEX-MEX strains were resampled in August when TEX-MEX was placed under factory production for increase in numbers.

Both the APHIS and TEX-MEX strains were reared separately in the factory and resampled in February when the APHIS strain was phased out. At the time these samples were taken a suitable trap for capturing living adult flies was not available. We therefore do not have direct estimates of gene frequencies in natural populations. However, a rough approximation of gene frequencies in the Texas and Mexican populations can be obtained from the May samples of the TEX and MEX lab strains which represented the end product of a series of carefully outcrossed matings. Selection and drift therefore were held at a minimum during the early development of these strains. The electrophoretically detectable forms of the enzymes studied were inherited as typical mendelian genes with each controlled by a single locus.

Electrophoresis

Adults were frozen alive on dry ice and held at −70°C until processing. Storage for 4 mo had little noticeable effect on the activity or mobility of most enzymes. However, LAP and most esterases began to show some reduced activity after prolonged freezing.

The horizontal starch gel electrophoresis technique was used to establish phenotypes (Poulik 1957, Beckman and Johnson 1964). Single adult homogenates were prepared following the method of Johnson (1966). Each frozen individual was homogenized with 4 drops of a solution consisting of 4 drops of 5 mM mercaptoethanol in 100 ml Poulik buffer (see below) with a Delrin® rod in a 6×13 mm well cut in a Delrin® plastic block. Each homogenate was absorbed onto a 9×6 mm piece of Whatman #1 filter paper which was inserted into slits cut into gels prepared by mixing equal parts of Electrostarch (Otto Hiller, Madison, Wisc.) and Connaught starch (Fisher Scientific, New York) at the concentration of 12 gm/100 ml of the appropriate buffer. Buffers and staining procedures were modified slightly from Shaw and Prasad (1970).

Buffer Systems

(1) for Esterase A (EST-A) and leucine amino peptidase (LAP) Tris-citrate (Poulik) gel buffer—4.6 gm trizma base and 0.53 gm citric acid/1 liter distilled water (Ph 8.6); electrode buffer—687 ml/n trizma base and 157 ml/N citric acid diluted to 2000 ml with water (Ph 8.0). (2) for Esterase-B (EST-B), phosphoglucomutase (PGM) and α-glycerophosphate dehydrogenase (α-GDH) Tris-HCl buffer—1.2 g trizma base and 4 drops concentrated HCl/1 liter distilled water (Ph 8.5); electrode buffer—18.2 g boric acid and 2.4 g sodium hydroxide/1 liter distilled water (Ph 8.4).

Stains and Substrate Buffers

Esterase stain—15 mg α-naphthyl acetate, 15 mg β-naphthyl acetate, 40 mg Fast Garnet: phosphate buffer—50 ml 0.2 n monobasic sodium phosphate, 10 ml 0.2 n dibasic sodium phosphate, 40 ml water (Ph 5.9). LAP stain—20 mg L-leucyl-β-napthyla-
mide HCl and 25 mg Black K salt, Tris-maleate buffer—50 ml 0.2 n tris acid maleate (23.2 g maleic acid, 24.2 g trizma base, in one liter distilled water (pH 3.8), 10 ml 0.2 n sodium hydroxide, 40 ml distilled water. α-GDH stain—200 mg α-glycerophosphate, 25 mg NAD, 20 mg NBT, 1 mg PMS. PGM stain—170 mg disodium glucose-1-phosphate, 0.75 mg dipotassium glucose-1, 6-diphosphate, 200 mg magnesium chloride, 10 mg NADP, 50 units glucose-6-phosphate dehydrogenase, 10 mg MTT, 1 mg PMS; PGI stain—25 mg fructose-6-phosphate dehydrogenase, 10 mg MTT, 1 mg PMS. DH substrate buffer for α-GDH, PGM and PGI—100 ml of 12.1 g trizma base and 8.5 drops concentrated HCl/1 liter distilled water (pH 8.4).

Gel slices were incubated in stain solutions at 37°C until bands became distinct, then fixed in water:methanol:glacial acetic acid (5:5:1).

**Results and Discussion**

As can be seen in Table 1, it is clear that the OLD APHIS strain, which had been reared continuously in the factory for over 40 generations, has a much lower level of genetic variation at the loci examined than found in the TEX-MEX population when it entered the factory. It was homozygous at both important Group I loci (α-GDH, and PGM) and in the Group II loci: EST-A, EST-B, and EST-6 alleles have been lost. A similar pattern appears in the NEW APHIS and PUERTO RICAN strains which had passed through approximately 20 generations in the factory at the time of sampling. These factory strains are essentially homozygous for PGM, while α-GDH occurs at very high frequency and may be slowly moving to fixation.

Although the genetic structure of the original population of both the OLD and NEW APHIS strains is not known, gene frequencies probably were similar to that of the TEX lab strain when it was first established as both originated from collections made in southwest Texas. At the time of sampling the level of polymorphism, therefore, they probably represent a loss of genetic diversity in the 2 APHIS strains during their tenure in the factory. As each cage housed around 70,000 flies it is unlikely that the loss can be attributed to drift. This is evident in systematic elimination of certain alleles in the series of samples taken before and after colonization of the TEX-MEX strain. This strain underwent profound and rapid changes that resulted in frequencies similar to those found in the APHIS strains.

The most dramatic shift occurs in the α-GDH locus as illustrated diagrammatically in Fig. 1. The α-GDH allele which is either fixed or nearly so in the APHIS strains is actually rare in the original TEX strain when it was founded and it is thus rare in natural populations in Texas as well. The same allele is more common in Mexico as it existed in the original Mexican strain at the frequency of 0.53.

After the 2 strains were hybridized during June and July and their hybrid progeny placed in the factory in August, the frequency of the α-GDH, was 0.31. Within 10 mo (about 15 generations) the frequency of α-GDH had risen to 0.74 in the factory. A similar though less dramatic trend was also noted in the PGM allele which shifted from 0.85–0.95 during the same period of time. These resulting frequencies are similar to those found in the

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* Could not be scored.
OLD and NEW APHIS strains. That all 3 strains would eventually establish similar frequencies in allelic forms of Group I enzymes indicates that the primary cause is directed selection rather than drift.

Shifts in gene frequency also were observed in the Group II loci. At EST-A there was a rather large increase in the EST-A_1 allele and a commensurate loss in both the EST-A_2 and EST-A_3 alleles resulting in frequencies similar to those found in the 2 APHIS strains. Although only minor shifts are apparent at the EST-B locus, frequency changes in the TEX-MEX strain eventually resulted in a pattern like the one found in the NEW APHIS strain. At the LAP locus no conspicuous shifts occurred, suggesting that this locus is not affected by the mass rearing program.

The frequency changes observed in 2 (TEX-MEX and MEX) of the 3 ARS laboratory strains maintained in much smaller population densities follows a different pattern than observed in factory colonies (Table 2). Initially ω-GDH exhibited a relatively high frequency of 0.533 in the MEX strain which dropped drastically to 0.21 within about 20 generations (Fig. 1). On the other hand, this same allele rose dramatically in the TEX strain from a negligible level of 0.005 to high frequency of 0.547. The change in allele frequency of the hybrid lab TEX-MEX strain parallels that of the lab MEX dropping from 0.310–0.074.

Although lab cages usually held about 500 flies, a reduction in population size sometimes occurred which could have resulted in considerable inbreeding and drift. Alternatively, the TEX and MEX laboratory flies derived from different geographic regions might be responding differently to similar selection forces present in the laboratory but not present in the factory.

Two other strains are available for comparison. The FLORIDA strain is descended from flies utilized during the successful 1950’s program which eradicated the screwworm from the southeastern United States (Smith 1960). Data is available for only 2 loci (ω-GDH and PGM) from this strain and sample size is small (N = 12). PGM may be fixed for allele PGM_2 while significant variation is still present at ω-GDH [f(1) = 0.625; f(2) = 0.375]. This strain, despite its long period of artificial maintenance has retained some variability, probably because it has never been reared under factory conditions. During and after the program in Florida,
Table 2.—Allele frequencies for 2 flight muscle enzymatic loci in small laboratory populations of the screwworm fly.

<table>
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<th>Type</th>
<th>Locus</th>
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<th>TEX</th>
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<td>Apr '74</td>
<td>May '73</td>
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<td>.533</td>
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<td>.995</td>
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<td>(N) 12</td>
<td>106</td>
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<td>105</td>
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<tr>
<td>I</td>
<td>PGM</td>
<td>1 .000</td>
<td>.000</td>
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<td></td>
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<td>(N) 12</td>
<td>106</td>
<td>95</td>
<td>101</td>
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</table>

this strain has been reared under conditions identical or similar to those of the laboratory strains reported above.

From the standpoint of performance of factory reared flies under natural conditions, the changes in Group 1 enzymes are probably the most significant. Evidence is accumulating that some allelic allozymes have subtle yet physiologically and ecologically significant differences of adaptive importance (Johnson 1974, Wills and Nichols 1973, Scandalios et al. 1972, Felder and Scandalios 1971, McKechnie et al. 1975, Ainsly and Kitto 1975, Minawa and Birley 1975). The recent studies of Miller et al. (1975) are of particular interest for they found that α-GDH allozymes from Drosophila melanogaster differed in three important parameters, namely temperature dependence of specific activity, temperature dependence of K_m, and reaction rate constancy for a physiological temperature range. The allelic forms of this key enzyme are therefore very sensitive to ambient temperatures and, as they point out, there is a good correlation between the environmental pattern encountered in natural populations and the kinetic differences in the enzymes. The principal component of variation observed in the spatial and temporal frequency clines at the α-GDH locus (Johnson and Schaffer 1973) is temperature. The same is probably true for the PGM locus but the kinetic studies on this enzyme are not yet available.

The rapid shift in gene frequency at the α-GDH and PGM loci during colonization and factory rearing is therefore most likely to be the direct outcome of selection for their allelic forms that function well under domestication but not necessarily under field conditions. The constant temperature of the room in which adults were maintained for maturation, mating and egg laying may well exert a strong selective advantage for one α-GDH over the other.

Preliminary kinetic studies on screwworm α-GDH (Kitto et al. 1976, Bush et al. 1976) support this view. While the two enzymes were found to have identical pH optima they differed in relative activity as the temperature was varied. In the range from 10–35°C the α-GDH_1 was 10–20% less active than α-GDH_2. The K_m for dihydroxyacetone phosphate also differed for the two enzymes and showed variation with temperature. In the range from 15–35° the K_m of the α-GDH_1 was found to be consistently higher that that for α-GDH_2.

Furthermore, recent trapping records now underway at Mission, Texas by the USDA-ARS team (J. Coppedge and E. Aherns, pers. com.) suggest that wild flies are active all day while factory flies appear later in the day (1:30 PM) and are most active in the afternoon. Mating in wild flies may therefore be completed before the factory flies become competent for sexual activity.

Rapid changes in laboratory populations appear to be common in many mass reared insects. The phenomenon has been repeatedly observed in Drosophila (Dobzhansky 1970) and other organisms (MacKauer 1976) and recently Bush (1975) has reported very extensive genetic alterations of populations of the coding moth (Laspeyresia pomonella) that had been mass reared in various laboratories for different lengths of time.

The use of allozymes to genetically track ecologically significant loci at the biochemical level provides a potentially useful tool in quality control programs. This is particularly true for a species of strong flying insect where changes in Group I enzymes may not be the only enzymes important to consider. Although only Group I enzymes of regulatory importance have been studied in the screwworm fly thus far, others involved in carbohydrate metabolism such as glycero-phosphate oxidase should be given attention. Furthermore, enzymes critical for normal lipid metabolism (e.g., β-hydroxyacyl-CoA dehydrogenase) and amino acid metabolism (e.g., proline dehydrogenase) might be worth detailed examination. The possibility that 2 allelic variants which produce only slight variations in fitness when they occur independently may have an extremely large synergistic effect when acting together warrants consideration.

From the practical standpoint, the information obtained as a result of our investigation can be put to use in the Sterile Screwworm Release Program. Factory conditions may be altered to reduce discrepancies between fitness of flies adapted for natural conditions and those produced in the factory. Alternatively, new strains may be introduced at appropriate times to improve the quality of factory released flies in the field. Also knowledge of the
existence of geographic races and clines that reflect local adaptations are important factors to consider in designing future sampling programs for establishing new production lines.

**Acknowledgment**

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