Screwworm Eradication: Inadvertent Selection for Noncompetitive Ecotypes During Mass Rearing

Abstract. *The rapid fixation of a rare allelic form of α-glycerol phosphate dehydrogenase is related to a loss of competitive ability in nature of factory-reared screwworm flies. The increase in frequency results from selection for survival under conditions of domestication and rapid development at high constant temperatures in the factory.*

The screwworm *Cochliomyia hominivorax* caused serious losses to the livestock industry of the southwestern United States until 1962. At that time, a nationwide program was launched to eliminate all infestations in this country and to suppress the pest population in northern Mexico to such low levels that few screwworms would migrate northward. This was accomplished by diligently treating the wounds of all infested animals with insecticidal smears and by releasing billions of adult screwworm flies that had been exposed to sexually sterilizing doses of gamma rays. (The gamma rays induce dominant lethal mutations in the sperm.) Since eggs fertilized by such sperm fail to hatch, the reproductive capacity of the wild population can be greatly reduced if the ratio of the sterile flies to the wild population is more than 10 : 1. However, such suppression occurs only if the released males are able to successfully locate and inseminate wild females.

From 1962 to 1971 the Screwworm Eradication Program conducted by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) protected the Southwest from the screwworm. However, infestations jumped from about 473 in 1971 to 94,551 during 1972 (*1*). Although the number of cases dropped in subsequent years (9,000 in 1973; 7,272 in 1974; and 17,568 in 1975), infestations have never returned to the pre-1972 levels for reasons not yet fully understood (*2*).

Calman and Smith (*3*) suggested that intense selection pressures generated by the release of more than 10 billion irradiated adult screwworms a year may have resulted in the rapid evolution of a wild strain that no longer mates readily with released flies; at the same time, 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 males.

No economical method for testing the mating propensity of factory-reared flies under natural conditions has 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 are no longer suppressed to the same degree as in the past.

One important aspect of the fly's biology that is related to mating activities is its great flight capacity. Adults have been captured as far as 180 miles (1 mile = 1.6 km) from a release point, and many flies travel at least 10 miles as adults (*4*). Because natural population densities are normally low (approximately 100 to 200 per square mile) (*5*) and factory flies are distributed by aircraft along parallel flight paths up to 10 miles apart, released flies must be able to disperse rapidly and be active throughout the day if they are to find suitable environmental conditions for survival, courtship, and mating in order to remain competitive with wild flies.

At the time of the outbreak in 1972, the mass rearing methods in use 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 Puerto Rico and the old and new "APHIS" strains (*6*) were held in large cages at 25° ± 1°C in total darkness (*7*). Each cage was subdivided by many large sheets of newsprint suspended from the cage ceiling to increase the surface area and to 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 that flew only short distances. In addition, these flies were reared at a relatively high constant temperature and were thus not exposed to the highly variable weather conditions encountered by released individuals in nature. Selection for maintaining adequate flight muscle activity to ensure normal dispersal in nature was therefore relaxed. A further premium was placed on rapid development from egg to pupa at high constant temperatures; short-generation flies were positively selected in order to promote efficient plant operation regardless of their overall suitability as competitors against wild flies.

Flight muscle enzymes that directly or indirectly regulate energy flow through metabolic pathways appear to be particularly sensitive to selection (*8*). Therefore we examined the effects of the factory and laboratory environments on two allelic forms of α-glycerol phosphate dehydrogenase (α-GDH) [glycerol-3-phosphate dehydrogenase (E.C. 1.1.1.8)] (*9*) by gel electrophoresis (*10*) in several strains of the fly colonized from various areas of Texas and northern Mexico (*6*). This key enzyme in strong flying insects governs the transfer of reducing equivalents from cytoplasmic reduced nicotinamide adenine dinucleotide (NADH) to the mitochondrial electron-transport chain by way of the glycerol phosphate shuttle (*11*). In *Drosophila* the amount of α-GDH present in flight muscle is highly correlated with flying ability (*12*), and mutants that lack α-GDH activity cannot fly (*13*). By controlling the concentrations of nucleotide cofactors, α-GDH may indirectly regulate metabolic rates (*8*).

The Puerto Rico, old APHIS, and new APHIS strains reared continuously in the factory for 25, 40, and 20 generations, respectively, are fixed or nearly fixed for the α-GDH$_1$ allele (Table 1). Although the genetic structure of the original population of these strains is not known, gene frequencies (f) of α-GDH$_1$ in the old and new APHIS strains were probably similar to those found in the "Tex" laboratory strain (f = .01) (all of which originated from flies drawn from southwestern Texas). The Puerto Rico strain may have been more like the more warm-adapted "Mex" strain (f = .53) when it was first established. As each factory cage housed between 40,000 and 70,000 flies depending on the strain (*6*), it is unlikely that the loss of the α-GDH$_2$ allele in the factory can be attributed to drift.

The systematic elimination of α-GDH$_2$ is evident in a series of samples taken before and after colonization of the Tex-Mex strain (Fig. 1). This strain, synthesized by outcrossing the Tex and Mex laboratory strains and reared under a diurnal light cycle and at lower cage densities (*6*), underwent profound and rapid shifts in gene frequencies paralleling those that probably occurred in the Puerto Rico and APHIS strains before this study was initiated.

After the two strains were hybridized during June and July and their hybrid progeny were placed in the factory in August, the frequency of the α-GDH$_1$ was .31. Within 10 months (about 15 generations) the frequency of α-GDH$_1$ had risen to .74 in the factory. The resulting fre-

quencies are similar to those found in the old and new APHIS factory strains. That all four factory strains originating at different times and from different areas would eventually establish similar α-GDH$_1$ frequencies in the factory indicates that the primary cause is directed selection rather than drift.

Unlike that of the factory flies, the frequency of α-GDH$_1$ in two (Mex and Tex-Mex) of the three Agricultural Research Service (ARS) laboratory strains (also reared at 25°C) declined during the course of this investigation. In this case, drift rather than selection appears to be an important factor. Although cage populations average 500, occasional reduction to low numbers occurs and results in considerable inbreeding.

Such drift may be responsible for the differing responses of the laboratory Tex and the Mex and Tex-Mex strains, although selection cannot be entirely ruled out. The frequency shift may represent the response of different coadapted gene pools to the ARS laboratory environment. It seems unlikely, however, that selection could overcome the effects of considerable drift that must occur in the small laboratory populations.

Evidence is accumulating that some allelic allozymes [different enzyme forms produced by different alleles at the same locus (*14*)] have subtle but physiologically and ecologically significant differences of adaptive importance (*8, 15*). The recent studies of Miller *et al.* (*16*) are of particular interest for they found that α-GDH allozymes from *Drosophila melanogaster* differed in three important parameters: (i) temperature dependence of specific activity, (ii) temperature dependence of the Michaelis constant (K_m), and (iii) reaction rate constancy for a physiological temperature range. Furthermore, in natural populations the principal component of variation in the spatial and temporal frequency clines of this key enzyme is temperature (*16,17*).

The rapid shift in the frequency of the α-GDH$_1$ allele during colonization and factory rearing is therefore likely to be the direct outcome of selection for an allelic form that functions well under domestication but not necessarily under field conditions. The constant temperature of the room in which the adults are maintained for maturation, mating, and laying eggs exerts a strong selective advantage for α-GDH$_1$.

Our preliminary kinetic studies on the screwworm α-GDH allelic variants support this view (*9, 18*). While the two enzymes showed identical *p*H optima, they differed in relative activity as the temperature was varied. From 10° to 35°C, the α-GDH$_1$ was 10 to 20 percent less active than α-GDH$_2$. The K_m for dihydroxyacetone phosphate also differed for the two enzymes and showed variation with temperature. From 15° to 35°C, the K_m of the α-GDH$_1$ was consistently higher than that for α-GDH$_2$.

Furthermore, a field study at Mission, Texas, by the U.S. Department of Agriculture–Agricultural Research Service team in which all adults arriving on wounded animals were captured and examined, indicated that wild flies were active throughout the day from early morning to late afternoon, but factory flies appeared later, usually in early afternoon (*19*). Mating in wild flies may therefore be completed before the factory flies become competent for sexual activity. These findings and the results of our study on gene frequency shifts during mass rearing indicate that heterozygous and possibly homozygous α-GDH$_2$ flies may be competitively superior to homozygous α-GDH$_1$ flies.

Rapid changes in the gene frequencies of laboratory populations appear to be common in mass-reared insects. The phenomenon has been repeatedly observed in *Drosophila* (*20*), and Bush (*21*) has reported extensive alterations in populations of the codling moth (*Laspeyresia pomonella*) that had been mass-reared in various laboratories for different lengths of time.

The use of allozymes to genetically track ecologically and behaviorally significant loci at the biochemical level provides a useful tool in quality control pro-

Table 1. Frequency of the α-GDH$_1$ allele of α-glycerol phosphate dehydrogenase in the U.S. Department of Agriculture–Agricultural Research Service laboratory and the Animal and Plant Health Inspection Service factory. Sample size is in parentheses. The Tex-Mex strain was synthesized by hybridizing Tex and Mex strains. A sample taken from this population was used to establish the laboratory and factory colonies. Laboratory strains of Tex and Mex flies represent close approximations of gene frequencies found in wild populations.

Strain	Frequencies at different sampling dates				
	1973		1974		
	May	August	February	April	June
Puerto Rico factory	.91 (27)	Phased out			
Old APHIS factory	1.00 (31)	Phased out		Phased out	
New APHIS factory	.89 (82)	.90 (122)	.91 (133)	Phased out	
Tex-Mex factory		.31 (121)	.65 (113)	.60 (108)	.74 (96)
Tex laboratory	.01 (106)			.55 (95)	
Mex laboratory	.53 (105)			.21 (107)	
Tex-Mex laboratory		.31 (121)		.07 (108)	

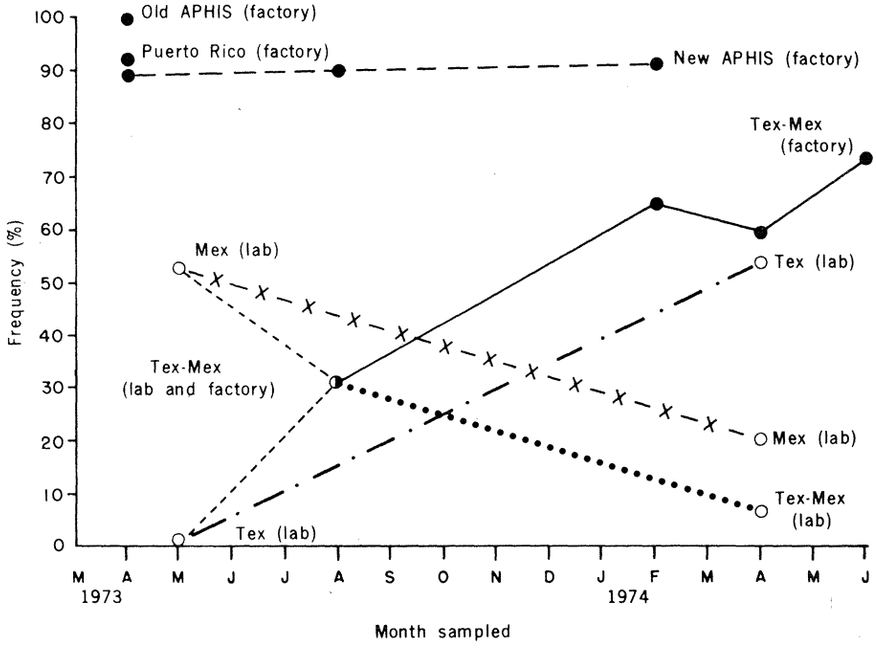

Fig. 1. Changes in the frequency of the α-GDH$_1$ allele during colonization. Open circles represent small laboratory populations; closed circles represent large factory populations.

grams of mass-reared insects. In the Sterile Screwworm Release Program, factory conditions may be altered to improve flight capacity under natural conditions. Alternatively, new strains may be synthesized at appropriate times to meet changing environmental conditions and to maintain high quality of factory-released flies in the field. Knowledge of the existence of geographic races and clines that reflect local adaptations are also important factors to consider in designing future sampling programs for establishing new production lines.

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6. The old APHIS strain used during the 1972 outbreak originated from larvae collected in early 1971 at several sites in northern Mexico. New APHIS factory-produced flies used in the 1973 eradication campaign originated from several thousand larvae (reared in wounds) collected in August 1972 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. The Tex-Mex strain was synthesized by outcrossing equal numbers of Mex adults reared from six egg masses collected on wounded animals in three northern Mexican states with progeny of 18 egg masses collected on wounded cattle from 12 southern Texas counties (Tex). Approximately 35,000 to 40,000 Tex-Mex factory adults were held per cage under diurnal light conditions [11 hours at 4.8 lux with 1 hour at 0.0048 lux (dawn and dusk periods in 24 hours)]. 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. Rough approximations can be obtained from the May sample of the Tex and Mex populations, as these strains represented the end product of a series of carefully outcrossed matings. Factory strains were allowed to lay eggs at the age of 4 to 5 days, and laboratory strains at the age of 7 to 9 days.
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Side Chain Metabolism of 25-Hydroxy-[26,27-^{14}C]vitamin D_3 and 1,25-Dihydroxy-[26,27-^{14}C]vitamin D_3 in vivo

Abstract. *Radioactive CO_2 was detected in expired air after the administration of 25-hydroxy-[26,27-^{14}C]vitamin D_3 to vitamin D–deficient hypocalcemic rats; $^{14}CO_2$ was also detected after the administration of 1,25-dihydroxy-[26,27-^{14}C]vitamin D_3 to rats raised on the same diet. Nephrectomy totally abolished $^{14}CO_2$ formation after administration of 25-hydroxy-[26,27-^{14}C]vitamin D_3, but not after the administration of 1,25-dihydroxy-[26,27-^{14}C]vitamin D_3. The production of $^{14}CO_2$ commenced within 4 hours after injection of 1,25-dihydroxy-[26,27-^{14}C]vitamin D_3, suggesting a possible relevance of this reaction to the function of 1,25-dihydroxyvitamin D_3. These results at least demonstrate a new metabolic pathway of vitamin D_3 metabolism involving the oxidation of a portion of the side chain of 1,25-dihydroxyvitamin D_3 to CO_2.*

It is now clear that, physiologically, 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] or a further metabolite is the metabolically active form of vitamin D_3 (*1*). Although alternate metabolites derived from 25-hydroxyvitamin D_3 (25-OH-D_3) are known (*2*), little is known concerning the further metabolism of 1,25-$(OH)_2D_3$. At 6 and 12 hours after the administration of 1,25-$(OH)_2$-[26,27-^{3}H]D_3 to rats and chicks, no polar metabolites were detected in the lipid extracts of intestine and bone (*3*, *4*). Since at that time both organs had responded to 1,25-$(OH)_2D_3$, it appeared likely that 1,25-$(OH)_2D_3$ is not further metabolized before it functions. However, radioactivity was detected in water-soluble material (*3*, *5*) and, furthermore, the overall recovery of the ^{3}H in the animals was considerably less than 100 percent, suggesting loss of the label as ^3H_2O. To examine this question fully, 25-OH-[26,27-^{14}C]D_3 was synthesized chemically by methods described for 25-OH-[26,27-^{3}H]D_3 (*6*). The 25-OH-[26,27-^{14}C]D_3 was converted to 1,25-$(OH)_2$-[26,27-^{14}C]D_3 as described (*7*). Both ^{14}C-labeled compounds are fully biologically active, giving antirachitic activity of 180 ± 20 unit/μg and 410 ± 30 unit/μg for the 25-OH-[26,27-^{14}C]D_3 and 1,25-$(OH)_2$-[26,27-^{14}C]D_3, respectively, when tested as described by Tanaka *et al.* (*8*). Furthermore, the 1,25-$(OH)_2$-[26,27-^{14}C]D_3 cochromatographs exactly with synthetic 1,25-$(OH)_2D_3$ (*9*), on high-pressure liquid chromatography, which separates all known metabolites of vitamin D_3 (*10*) (Fig. 1). The possible metabolism of these compounds to $^{14}CO_2$ was then examined in search of a pathway resulting in loss of the ^{3}H and ^{14}C from the 26 and 27 positions.

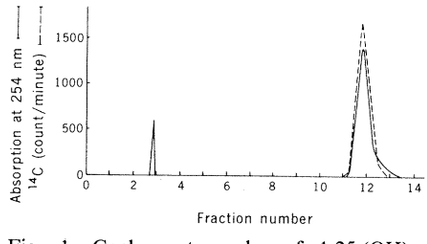

Fig. 1. Cochromatography of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 with crystalline 1,25-$(OH)_2D_3$ (Hoffmann–La Roche Inc.) on a high-pressure liquid chromatograph (Dupont 830) with a fine-grain silicic acid column (*10*) and a solvent of 15 percent isopropanol in Skellysolve B. 1,25-$(OH)_2$-[26,27-^{14}C]D_3 was prepared enzymatically from 25-OH-[26,27-^{14}C]D_3 and mixed with 20 ng of crystalline 1,25-$(OH)_2D_3$; the mixture was injected into the high-pressure liquid chromatograph. The ^{14}C was determined by liquid scintillation with the crystalline 1,25-$(OH)_2D_3$ detected by a monitor at 254 nm.

Male, albino weanling rats (Holtzman) were fed a vitamin D–deficient diet con-