PRELIMINARY OBSERVATIONS ON GENETIC VARIATION IN THREE COLONIES OF *MUSCA DOMESTICA* (DIPTERA: MUSCIDAE) ISOLATED FROM CENTRAL ALBERTA

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ABSTRACT

Three colonies of house flies, Musca domestica *L., were established using flies collected from a chickenbarn, a cattle feedlot, and an enclosed pigbarn. (The latter population bred year-round and was insecticide resistant.) Banding patterns on polyacrylamide gel electrophoresis, of heads and thoraces, of adults from these colonies indicated that malic acid dehydrogenase and* α-glycerophosphate dehydrogenase were monomorphic; tetrazolium oxidase, octanol dehydrogenase, and alkaline phosphatase were polymorphic and each was controlled by a locus on an autosome; and glucose-6-phosphate dehydrogenase was polymorphic and controlled by a locus on the X-chromosome. Each of the polymorphic loci had two alleles. Allele frequencies indicated that the colonies were genetically very similar and gave no firm evidence that the insecticide resistant population was genetically isolated from the other populations.

RÉSUMÉ

Trois colonies de mouches domestiques, Musca domestica *L., ont été établies à partir de mouches prélevées dans un poulailler, un enclos à bétail et une porcherie (la population habitant la porcherie se reproduisait toute l'année et était résistante aux insecticides). Les séries de bandes révélées par l'électrophorèse d'homogénats de têtes et de thorax d'adultes sur gel de polyacrylamide montrent que la déshydrogénase de l'acide malique et la déshydrogénase de l'*α-glycérophosphate sont monomorphiques; l'oxydase du tétrazolium, la déshydrogénase de l'octanol et la phosphatase alcaline sont polymorphiques et chacune est sous le contrôle d'un locus situé sur un autosome; la déshydrogénase du glucose-6-phosphate est polymorphique et est sous le contrôle d'un locus situé sur le chromosome X. Chaque locus polymorphique a deux allèles. Les fréquences des allèles indiquent que les colonies sont génétiquement très semblables mais elles ne fournissent pas d'évidence décisive à l'effet que la population résistante aux insecticides est génétiquement isolée des autres populations.

INTRODUCTION

House flies in a colony isolated in 1979 from an enclosed pigbarn near Calmar Alberta, southwest of Edmonton, were resistant to four organophosphate and three pyrethroid insecticides (Harris *et al.* 1982). House flies from the surrounding populations were apparently susceptible to insecticides since they were controlled by insecticide applications. If this resistance were genetically determined by recessive alleles one would expect that the population must be at least partially isolated from the surrounding house fly populations, otherwise the genes for resistance would become diluted due to outbreeding. One way to investigate the extent to which the population in the pigbarn is isolated from the surrounding populations is to compare the allele frequencies at several loci in the resistant population with the corresponding

allele frequencies in the surrounding populations. If the allele frequencies in the pigbarn population are different from those in the other populations in the region this would indicate that the former is, to some extent, genetically isolated from the latter.

The objective of this study was to determine whether the insecticide resistant population of *Musca domestica* L., in the pigbarn referred to above, was isolated from other house fly populations in the area by comparing allele frequencies in colonies established from these populations using polyacrylamide gel electrophoresis.

Ideally such a study should be made by examining flies collected at each of the sites studied, and by examining a large number of loci in each population. However, because this project was part of a course which had to be carried out in the winter months and because no previous work had established methods for storing house flies for subsequent electrophoretic study, I decided to establish colonies from the pigbarn and from two other nearby locations and to electrophorese their descendants. Because of the dearth of information about electrophoresis of *M. domestica* and because of time restraints only six loci were examined.

MATERIALS AND METHODS

Three populations of house flies, *Musca domestica* L. were sampled August 25, 1981. Colony 1 was established using 300 to 400 flies collected from a pig barn, colony 2 using 50 to 100 flies from a chicken farm, and colony 3 using about 150 flies from a feedlot operation; all occurred within an 8 km radius in the Calmar area southwest of Edmonton Alberta. The pig barn population was in an enclosed structure and was able to reproduce throughout the year. Insecticides were intensively used in this barn and a chronic problem with house flies occurred there. At the time the flies were collected unsuccessful attempts were being made to suppress the population using pyrethroid insecticides. The other two populations were not known to reproduce throughout the year and were presumed to overwinter as hibernating adults or to have been re-established each spring by adults immigrating from winter refugia. Insecticides were occasionally (and successfully) used at the chicken farm and the feedlot, and house flies were not particularly troublesome at either site.

The three colonies were maintained in the culture room at the University of Alberta, Department of Entomology and were used over several generations. The medium for rearing larvae consisted of 100 ml bran, 100 ml wood chips, 25 ml milk powder and 100 ml water. Adults were fed a mixture of dried eggs and sugar, and water was dispensed *ad lib.* through a cotton swab. Eggs were collected every two weeks. Relative humidity in the room varied from 30 to 85%. Lights in the room were controlled on a 14 hour light:10 hour dark cycle.

The procedure and apparatus used for polyacrylamide gel electrophoresis were those described by Gooding and Rolseth (1982), with the following modifications. All of the electrophoretic runs used a 7% gel at pH 8.9. The head and thorax from each fly were homogenized together and there was enough homogenate from each house fly to do two runs. Each gel was stained for one or two of the following enzymes: tetrazolium oxidase (TO), octanol dehydrogenase (ODH), alkaline phosphatase (ALKPH), α-glycerophosphate dehydrogenase (α-GPD), glucose 6-phosphate dehydrogenase (G6PD), and malic acid dehydrogenase (MDH) using the methods outlined by Gooding and Rolseth (1982). Four to five months after colonizing the flies, comparisons were made using a mimimum of 17 adults from each colony. The comparisons were facilitated by treating samples from two colonies on each gel.

The taxonomic identity of the flies was confirmed by Dr. G.C.D. Griffiths, and voucher specimens were deposited in the Strickland Museum, Department of Entomology, University of Alberta.

RESULTS AND DISCUSSION

Electrophoretic patterns and genetic interpretation

For TO, ODH, and ALKPH each fly had either one band or three bands (Fig. 1). This is interpreted as indicating that for each enzyme there is one locus with two alleles, and that the active form of each enzyme is a dimer. Heterozygotes were found in both sexes, indicating that the loci for these enzymes are on autosomes. As far as I am aware this is the first report of an electrophoretic study of ALKPH in *M. domestica*. On polyacrylamide gel electrophoresis two zones staining for TO were reported by McDonald *et al.* (1975). The slower migrating zone appeared to be monomorphic, and the faster migrating zone was controlled by a locus (*To2*) having two alleles (McDonald *et al.* 1975). On the basis of electrophoretic mobility and banding patterns, it is likely that the locus I studied (*To*) corresponds to locus *To2* described by McDonald *et al.* (1975). These authors reported four zones on polyacrylamide gels staining for ODH. Three of the zones were either monomorphic or stained diffusely and were not consistently readable. The locus *Odh1* produced consistently readable bands and had two alleles. It was established, by breeding experiments, that heterozygotes had three bands. Based upon electrophoretic mobility and banding patterns, it is likely the *Odh* locus studied here corresponds to *Odh1* described by McDonald *et al.* (1975).

Each fly had one or three G6PD bands (Fig. 1) and this is interpreted as indicating that this enzyme is controlled by one locus with two alleles, with the active form of the enzyme being a dimer. The locus for this enzyme appears to be on the X-chromosome since no heterozygous males were found (Table I). This enzyme is also known to be on the X-chromosome in tsetse flies (Gooding 1983).

MDH and α-GPD bands did not vary (Fig. 1). This indicates that each enzyme is controlled by a single locus but its location is unknown since these enzymes were monomorphic.

With the exception of G6PD, phenotype frequencies within each house fly colony indicated that each colony was in Hardy-Weinberg equilibrium at the loci examined (Table I).

Intra-colony variation

A commonly used measure of genetic variation within a population is heterozygosity. This was estimated in each colony (from data in Table II) as the expected average frequency of heterozygotes per locus (H): colony 1, $H = 19.8 \pm 6.8\%$; colony 2, $H = 10.0 \pm 4.5\%$; and colony 3, $H = 10.9 \pm 6.6\%$. (Values for H and the S.D. were calculated using equations 6.5 and 6.6 from Nei [1975].) Although colony 1 was slightly more heterozygous than colonies 2 and 3, the values obtained for H are all comparable to the average values seen in other insect populations. (For examples of H values in other invertebrates see Dobzhansky *et al.* [1977, Table 2-9] or Ayala [1982, Table 2.11], and for examples of values found in colonies of tsetse flies see Gooding [1982].)

Field collected *M. domestica* from Mission, Texas had three alleles present at a TO locus and two alleles at an ODH locus (McDonald *et al.* 1975) but the frequencies of these alleles and the heterozygosity at these loci were not reported. Genetic variations of TO and ODH have been studied in two populations collected near Fargo, North Dakota (McDonald and Johnson 1976). Both populations had two alleles at the *To* locus (with the commonest allele being the

same in each population and having frequencies of 88% and 97%). One population had three *Odh* alleles, with frequencies of 4%, 76%, and 20%, while the frequencies of the same alleles in the second population were 0%, 82%, and 18% respectively (McDonald and Johnson 1976). There appear to be no published studies of genetic variation in natural or laboratory populations of *M. domestica* involving any of the other enzymes which I studied. Variation in lactic acid dehydrogenase has been studied in several natural populations of *M. domestica* (Agatsuma and Takeuchi 1976, 1978a, 1978b) and variation in esterases in several strains of house fly has also been reported (Velthuis and van Asperen 1963, Narang *et al.* 1976). Breeding experiments demonstrated hidden heterozygosity on chromosome 3 in a house fly population near Fargo, North Dakota and it was estimated that 23.2% of the individuals carried one or more lethal alleles on chromosome 3 (McDonald and Overland 1974). Using polyacrylamide gel electrophoresis, allele frequencies were determined at six loci and variations, but not allele frequencies, were reported at two other loci in two natural populations of house flies collected near Fargo, North Dakota (McDonald and Johnson 1976). The latter study and the present report seem to be the only quantitative estimates of genetic variation in natural populations or recently isolated colonies of *M. domestica*.

Inter-colony comparisons

The overall genetic similarity of two populations may be estimated from allele frequencies in those populations by using any of several indices. Using the allele frequency data in Table II and the methods of Nei (1972, 1975) the mean genetic identity (I) of the pairs of colonies was estimated to be as follows: I(1:2)=0.9937, I(2:3)=0.9836, I(1:3)=0.9858. These values indicate that there were only slight differences between the three house fly colonies and that it is colony 3 (rather than colony 1) which is most different from the other two colonies.

If each of the colonies were established from the same population, and if the allele frequencies within the colonies had not changed due to selection or drift during colonization, one would expect the data to indicate that the population, from which the colonies were established, would be in Hardy-Weinberg equilibrium at each locus. Since the loci for MDH and α-GPD were monomorphic these loci can not be used in such a test. Nor can the data for G6PD be used since each of the colonies was not in Hardy-Weinberg equilibrium at this locus. For the three enzymes whose loci are on autosomes, analysis of the pooled data indicates that all three are in Hardy-Weinberg equilibrium: for TO $\chi^2=0.0297$, for ODH $\chi^2=0.0052$, for ALKPH $\chi^2=0.1042$; all χ^2 values have been calculated with Yates correction for 1 d.f.

Comparing the number of gene products observed in each of the colonies (Table 2) indicated that the three colonies were not significantly different for ODH ($\chi^2=5.8387$, 2 d.f.) or ALKPH ($\chi^2=5.3082$, 2 d.f.) For TO there were significant differences among the colonies ($\chi^2=7.4131$, 2 d.f., $0.01 < p < 0.025$), and this is largely attributed to the absence of the fast allele from colony 2. There were significant differences in the numbers of each type of G6PD observed in the three colonies ($\chi^2=13.3703$, 2 d.f.) but the significance of this is difficult to interpret since the colonies were not in Hardy-Weinberg equilibrium at the locus for G6PD.

As indicated above, colony 1 had a greater heterozygosity per locus than did either of the other colonies; the mean for the three colonies was 13.6%. Pooling the phenotype data from all three colonies indicated that the expected average frequency of heterozygotes per locus was 15.6%. These figures indicate that only 13% of the total variation is attributable to variation between colonies (see Hartl 1980).

General discussion

For reasons stated in the Introduction this study used colonized, rather than field collected, flies. A problem with this approach is that there were opportunities for sampling errors, genetic drift, selection, and inbreeding in the colonies. The colonies were established with reasonably large samples in an attempt to minimize sampling errors at that time. The heterozygosity observed in each colony was comparable to what is seen in naturally occurring populations of insects, indicating that inbreeding had not been severe. It is possible that during the four to 12 generations of colonization there could have been selection or drift which resulted in the colonies becoming more similar to each other than were the natural populations from which they were established. But such an event does not seem likely considering the level of heterozygosity in the colonies.

Overall, the data offer no firm evidence that the colonies were not isolated from the same population. Therefore I tentatively conclude that the insecticide resistant population in the pig barn was either not effectively isolated from the surrounding populations or if it were isolated, the isolation had not been for sufficient time to permit genetic differentiation, at the loci studied, of this population by either drift or selection.

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Table I
Phenotypes observed in three house fly colonies.

Enzyme[1]	Colony	s/s	s/f	f/f	H-W[2] χ^2
TO	1	50	9	1	0.746
		(.833)[3]	(.150)	(.017)	
	2	40	0	0	N.C.[5]
		(1.00)	(0.00)	(0.00)	
	3	50	9	0	0.355
		(.847)	(.153)	(0.00)	
ODH	1	2	17	31	0.011
		(.040)	(.340)	(.620)	
	2	0	3	14	0.103
		(.000)	(.176)	(.824)	
	3	2	13	13	0.181
		(.071)	(.464)	(.464)	
ALKPH	1	11	5	1	0.313
		(.647)	(.294)	(.059)	
	2	14	5	0	0.341
		(.737)	(.263)	(0.00)	
	3	17	1	0	0.000
		(.944)	(.056)	(0.00)	
G6PD[4]	1(M)[6]	10	0	17	
		(.370)	(.000)	(.630)	
	1(F)[6]	1	4	15	1.301
		(.050)	(.200)	(.750)	
	2(M)	4	0	16	
		(.200)	(.000)	(.800)	
	2(F)	1	2	16	5.139
		(.053)	(.105)	(.842)	
	3(M)	1	0	19	
		(.050)	(.000)	(.950)	
	3(F)	0	0	20	N.C.
		(.000)	(.000)	(1.00)	

[1]MDH and α-GPD were monomorphic, as indicated in figure 1.
[2]Calculated with correction for small sample size (Levene 1949).
[3]Genotype frequencies are given in parentheses.
[4]Genotype frequencies calculated according to Falconer (1981: 16-18).
[5]N.C., not calculated.
[6]M, male; F, female.

Table II.
Allele frequencies in three house fly colonies.

Enzyme	Allele[1]	Colony 1	2	3
TO	s	0.908 (109)[2]	1.000 (80)	0.924 (109)
	f	0.092 (11)	0.000 (0)	0.076 (9)
ODH	s	0.210 (21)	0.088 (3)	0.304 (17)
	f	0.790 (79)	0.912 (31)	0.696 (39)
MDH	c	1.000 (40)	1.000 (40)	1.000 (40)
α-GPD	c	1.000 (38)	1.000 (38)	1.000 (40)
ALKPH	s	0.794 (27)	0.868 (33)	0.972 (35)
	f	0.206 (7)	0.132 (5)	0.028 (1)
G6PD (F)[3]	s	0.150 (6)	0.105 (4)	0.000 (0)
	f	0.850 (34)	0.895 (34)	1.000 (40)
G6PD (M)[3]	s	0.370 (10)	0.200 (4)	0.050 (1)
	f	0.630 (17)	0.800 (16)	0.950 (19)

[1]Allele designation: s=slow, f=fast, c=common, i.e. only one band observed.
[2]Numbers of gene products observed are given in parentheses.
[3]F=female, M=male.

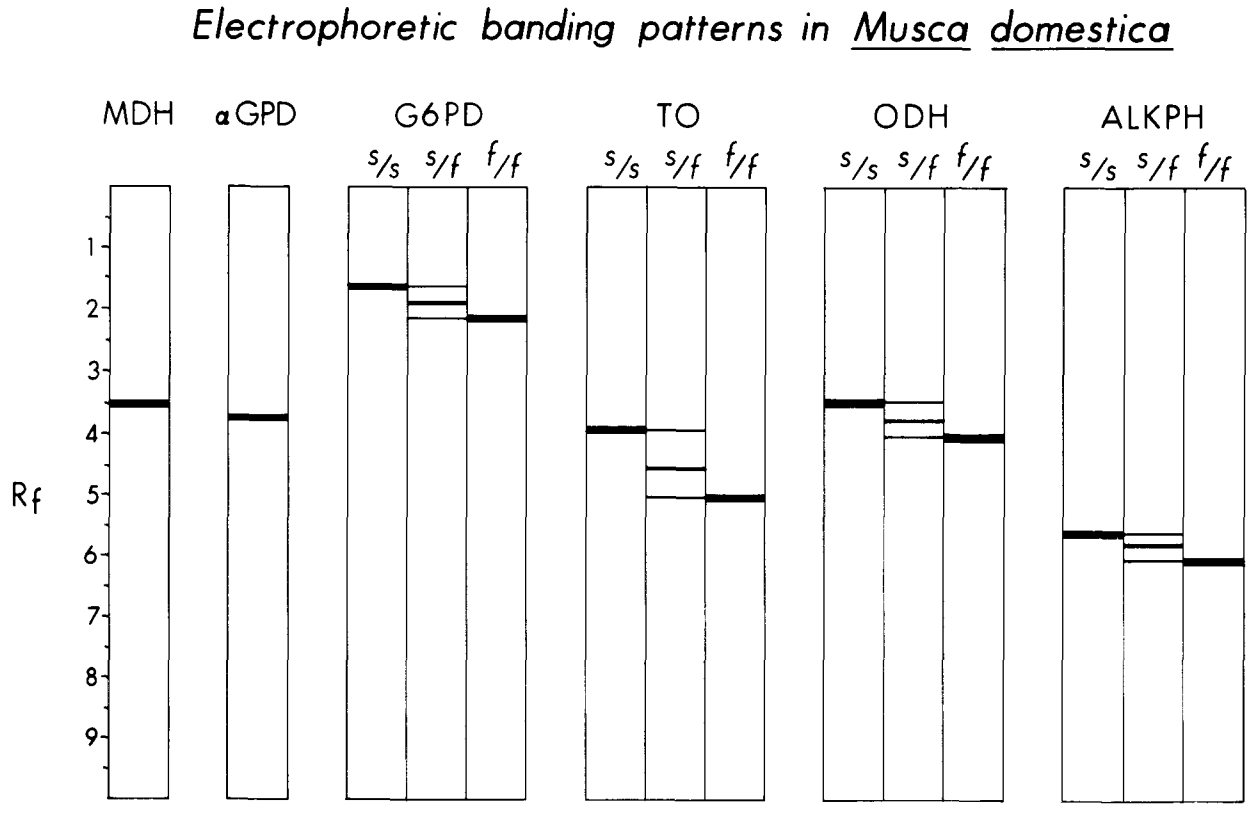

Figure 1. Diagram of the electrophoretic banding patterns observed in *Musca domestica*. MDH, malic acid dehydrogenase; α-GPD, α-glycerophosphate dehydrogenase; G6PD, glucose 6-phosphate dehydrogenase; TO, tetrazolium oxidase; ODH, octanol dehydrogenase; and ALKPH, alkaline phosphatase.