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SEASONAL AND REGIONAL VARIATION IN GENUS DROSOPHILA

BY

ZEINAB ABD EL MEGID EL HADIDI

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Approved by A.M. Omar ... Ibrahim
G.I. Nassrat ...
S.H. Hassanein

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Committee in charge

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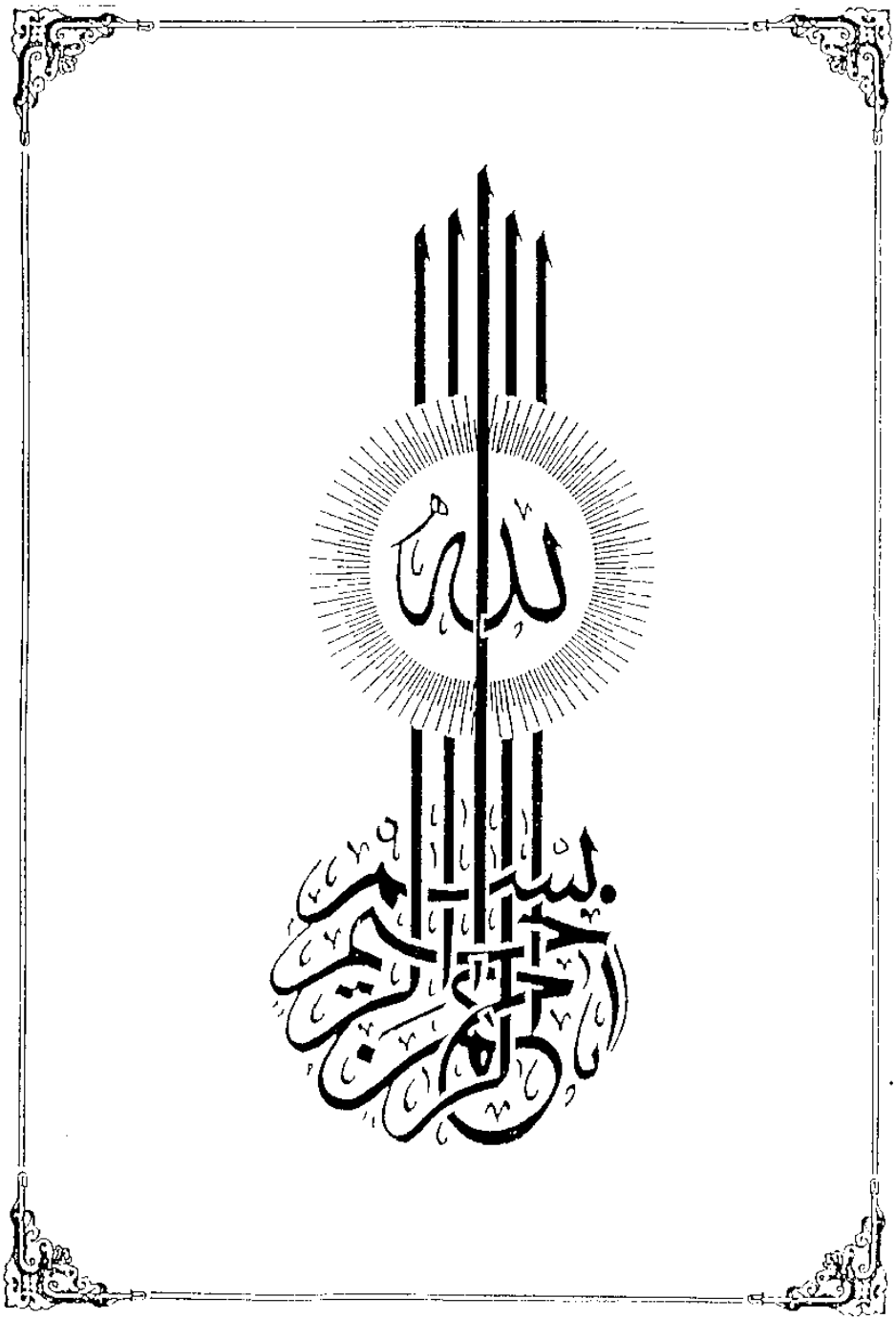

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I. INTRODUCTION

There are two basic problems in evolutionary genetics: (1) How big is the amount of genetic variation in natural populations? and (2) how big is the amount of genetic differentiation between populations? The first parameter measures the evolutionary potential of a population at a given time. Natural selection and precesses of random changes act on the existing genetic variability. The amount of genetic differentiation between population on the other hand reflects how much of evolutionary processes have taken place since the divergence of the respective populations from a common ancestral population unite. The genetic differentiation occurring in the process of speciation is of particular interest.

Gel electrophoresis in connection with other techniques permit quite well to know, on one side, the amount of genetic variation in populations, and, on the other hand, of the quantity of differentiation between populations. It has been argued, however, that most or all allelic variation detected by electrophoretic techniques may be adaptively neutral, and thus not subject to adaptive evolution (Kimura and Ohta, 1971). Yet, studies on the relationships between allelic frequencies in natural populations and various measurements of the environments in which these populations exist could shed some light on the problem. Kojima et al. (1972) investigated this question in Drosophila pavani, Rockwood-Sluss et al. (1973) in D. pachea, Tomaszewski et al. (1973), Salam et al. (1982) in D. melanogaster.

Especially for D. melanogaster it is known that this species is domesticated and regularly associated with human habitation, at least in the higher latitudes. The different enzyme and inversion frequencies in the various populations could thus be explained by the assumption that the number of ecological niches might be limited in temperate zones.

Yet, correlations between allelic frequency (genetic) patterns and environmental patterns were found for the other species too. In the studies mentioned above some relationship between the geographical location at which the populations were collected and the environmental characteristics as elevation, temperature and precipitation with genetic pattern were observed. It remains open, however, whether this is due to selective effects of the different environments or to non-selective, yet, location dependent effects, e.g., random gene flow as a result of drift and migration. Misapprehensions might occur if these two categories of acting forces are confused, as has been pointed out by Johnson and Schaffer (1973). However, when the genetic patterns are not correlated with geographic factors at all, it is practically not possible to differentiate between the two hypotheses.

Many analyses of natural populations of Drosophila melanogaster have demonstrated a large homogeneity of allozyme frequencies in all populations of the same geographical area (Berger, 1970; Franklin, 1971; Grossman, 1973; Johnson and Schaffer, 1973; Kojima et al., 1970; O'Brien and MacIntyre, 1969; Triantaphyllidis, 1973; Triantaphyllidis et al., 1973; Vigue and Johnson, 1973) and the influence of selection and migration on the determination of this homogeneity remains a highly debated question.

Other studies of various, widely separated D. melanogaster populations have revealed that, while nonrandom associations between alleles at different allozyme loci are uncommon, nonrandom associations between inversions and alleles at certain allozyme loci do occur rather frequently and may persist for a long time (Kojima et al. (1970), Mukai et al. (1971), Langley et al. (1974), Mukai et al. (1974), Mukai & Voelker, 1977). In spite of the extensive use of Drosophila melanogaster as an experimental organism in laboratory populations, relatively little is known about the patterns of inversion polymorphisms that exist in nature.

While a number of studies (Dubinin et al. 1937; Warters, 1944; Ives, 1947; Mourad and Mallah, 1960; Wetanabe et al. 1965, Watanabe, 1967; Yang and Kojima, 1972; Stalker, 1976; Ashburner and Lemeunier, 1976) have shown that inversions are generally not rare and may even reach high frequencies in some local populations, hence only a few studies deal with inversion frequency patterns over large geographical regions. Knowledge of inversion clines in Drosophila may be important in answering the general question whether their own maintenance is associated with that of allozyme variation. Linkage disequilibria between inversions and allozyme loci are not uncommon in this species Kojima et al. (1970), Mukai et al. (1974), Mukai and Voelker, (1977), Langley et al. (1974), Langley et al. (1977), Voelker et al. (1977).

Chromosomal polymorphisms are especially well studied in many Dipteran species since the presence of inverted chromosome segments can easily be observed and analysed in the giant chromosomes existing in these insects. A considerable number of investigations of inversion polymorphisms have been made by many population geneticists, mainly in order to obtain information about the genetic composition and architecture, or the dynamics of natural populations. Although Drosophila melanogaster is by far the genetically best studied of all Dipteran species, relatively little work has been done on its chromosomal polymorphism. The studies on karyotypic variability in natural populations have led so far to the conclusion that only a limited variety in the types of inversions exists. The "Standard" gene sequences of all chromosomes are always more common than any of the inverted sequences. Only six different autosomal inversions are frequent and cosmopolitan. X-chromosomal or pericentric inversions are very rare in natural populations (Warters, 1944; Ives, 1947; Mourad and Mallah, 1960; Watanabe, 1967; Yang and Kojima, 1972).

Chromosome inversion polymorphisms show different characteristics in different Drosophila species (Dobzhansky, 1970). Geographic frequency clines appear in D. pseudoobscura, D. robusta, D. flavopilosa, D. pachea, and D. subobscura (Dobzhansky, 1948; Stalker and Carson, 1948; Brncic, 1962, 1966; Ward et al., 1974; Pinsker et al. 1978). Cyclic and long-term temporal changes in inversion frequencies were observed in D. flavopilosa and D. pseudoobscura (Brncic, 1972; Anderson et al., 1975; Dobzhansky, 1943). Experimental studies and a bulk of observation data from natural populations have provided evidence that inversions are subject to natural selection (Dobzhansky, 1970).

A special type of information comes from studies dealing with the existence of non-random associations between different inversions of D. melanogaster. There are firstly, cases of significant disequilibria between linked inversions in natural populations (Alahiotis et al. (1976), Choi, (1977), Langley et al. (1977), Inoue and Watanabe, 1979). Secondly in some populations, exist a negative correlation between the occurrence of inversions heterozygosity in the second and third chromosomes; this may, in turn, contribute to an excess of individuals with an intermediate number of inversions (Stalker, 1976).

Since D. melanogaster has a world-wide spread, we can compare the distribution of variation between temperate and tropical populations as well as between central and marginal populations in this species. Further, since D. melanogaster has been well studied for other, non-enzymatic characters, it is possible to connect any observation on electrophoretic variation with other findings and conclusions.

The present work will report the results of studies on enzyme variability and on chromosomal polymorphisms in natural populations of D. melanogaster collected in Egypt and Germany. The allele frequencies for 16 different enzyme loci as well as the inversion frequencies were examined for 10 popualtions from these two countries.

II. REVIEW

A) Enzyme polymorphism

Electrophoretic techniques were applied to the estimation of genetic variation of isozymes in natural populations since 1966, when (Lewontin & Hubby) and (Prakash et al.) published, their works on Drosophila pseudoobscura. Electrophoretic techniques specially starch gel electrophoresis are currently being applied to many basic problems in population genetics and evolutionary biology. Thus, analyses of protein polymorphisms have focused for discribing the amount of allelic variation in many Drosophila species and for the distribution of this variation among component populations. In the following review only works on D. melanogaster and D. simulans are summarized. The symbols of the enzymes which are going to be mentioned in this dissertation are:

Acph	Acid phosphatase
Adh	Alcohol dehydrogenase
Aldox or Ao	Aldehyde oxidase
Ald	Aldolase
Aph	Alkaline phospatase
Amy	α -Amylase
Coe	Cholinesterase
Fum	Fumarase
Est	Esterase

Gi	Glucose isomerase
G-6-pdh	Glucose-6-phosphate dehydrogenase
Got-2	Glutamate oxaloacetate transaminase-2
Go	Glucose oxidase
∝ Gdh or ∝ Gpdh	∝ -Glycerol-3-phosphate dehydrogenase
Hk	Hexokinase
Idh	Isocitrate dehydrogenase
Ldh	Lactate dehydrogenase
Mdh	Malate dehydrogenase
Me	Malic enzyme
Odh	Octanol dehydrogenase
Pgm	Phosphoglucomutase
6Pgd = Pgd	6-phosphogluconate dehydrogenase
Sod	Superoxide dismutase
Xdh	Xanthine dehydrogenase

Berger, (1970) studied the variation at six enzyme loci [∝-Gdh, Adh, Mdh, Pgd, Ldh & G6pdh] in five sympatric populations of Drosophila melanogaster and D. simulans. Five of the six loci were highly polymorphic in D. melanogaster populations, while D. simulans was completely monomorphic at these loci. Studies of enzyme phenotypes in interspecific hybrids revealed hybrid enzyme pattern in four cases, suggesting homology of the relevant genes. In an interspecific comparison of variation at twelve enzyme

loci, a value of 34% allelic identity was calculated. In light of the substantial morphological similarity of the two species, this low level of allelic identity suggests that canalization may well transcend the species.

Trippa et al. (1970) collected a sample of 330 flies from a natural population of Drosophila melanogaster, at Nazzano, near Rome, Italy. Single-fly homogenates were examined for Pgm by an adaptation of the electrophoretic method of Spencer et al. (1964). The only natural population examined was polymorphic for this gene.

Charlesworth and Charlesworth, (1973) studied the linkage disequilibrium between five polymorphic enzyme genes (Est-6, Pgm, Xdh, Ao and Larval Aph) located on chromosome 3 of D. melanogaster. Out of the thirty possible tests for linkage disequilibrium between pairs of loci, two were significant at the 5% level and two at the 1% level. This result could not be reasonably ascribed to chance alone. The pairs of loci that had a significant correlation in one sample had higher average correlation in the other samples, this effect was highly significant statistically. There was no tendency for the high correlations to be associated with tightness of linkage between the loci concerned.

All five loci were involved in at least one significant effect. It was concluded that results are difficult to be explained on the neutral allele theory of protein polymorphism, but are consistent with the concept of selective control of allele frequencies.

Johnson and Schaffer, (1973) analysed the collections from U.S.A. of D. melanogaster for genetic variation at nine enzyme loci (Est-6, Est-c, Adh, α Gpdh, Acph, Mdh-D, Odh, Pgm and Aph). Allelic frequencies were determined, and comparisons between observed phenotypic proportions and those expected under equilibrium conditions were made. A significant tendency toward homozygote excess was noted. Relationships between patterns of genetic variability and patterns of genetic and environmental variability were examined by the method of principal components and correlation analysis. Several of the loci showed clinal patterns in gene frequencies, and overall there was an appreciable amount of genetic-genetic and genetic-environmental association.

Sing et al., (1973) tested selective neutrality of seven isozyme polymorphisms (Pgm, 6-Pgd, α Gpdh, Hex-3, Adh, Est-6 and Zw the abbreviation for G6pdh) using small

effective population size as an experimental design. Loci varied as to the degree of the decay of heterozygosity over 21 generations was retarded. Selection for heterozygote overdominance, was implicated for at least four of the seven loci. Of these Adh gave the largest heterozygote excess under the effect of inbreeding. An interaction between the small population size treatment and excess heterozygosity suggests that (1) the loci studied may be selectively neutral and linked to other loci which are under the influence of selection or (2) the selection coefficients for the loci studied are not independent from the background genotype. In either case there was evidence for selection for four of the seven enzymes studied. The problem of distinguishing the effect of a single marker from that of a chromosome segment was emphasized in this work. The identification of the genetic unit of selection is crucial to any interpretation of the meaning of enzyme polymorphisms.

Triataphyllidis, (1973) studied the variation at four gene loci (Est-c, Est-6, Acph-1 and 1-Aph) in sympatric populations of the sibling species Drosophila

melanogaster and D. simulans from Northern Greece. The allelic frequencies at two esterase loci showed a marked constancy in these populations over time. The frequencies of shared alleles in the two sibling species are significantly different. Studied at different developmental stages showed that the stages are characterized by the appearance and disappearance of three different groups of Aph bands. A new electrophoretic variant of larval Aph of Drosophila simulans was found, and was shown to be controlled by an autosomal codominant allele. Heterozygotes between this allele and the other known Aph allele showed a hybrid enzyme.

Schaffer and Johnson, (1974) demonstrated significant correlations between allelic frequencies and environmental variables in a number of insect species by multivariant techniques. Since many environmental variables show a strong relationship to geopraphic location and since gene flow between populations can also produce patterns of gene frequencies which are related to the geographic location, both selection and gene flow hypotheses are consistent with the observed correlations. The genetic variables can be corrected for geographic location and so for linear gene-flow patterns. If, after correction, the genetic variables still show