69-5411

ELLIS, Albert Temple, 1930-

AMINO ACID AND PEPTIDE METABOLISM AS INFLUENCED BY GROSS GENE REARRANGEMENTS IN DROSOPHILA PSEUDOOBSCURA.

University of Arizona, Ph.D., 1968
Biology-Genetics

AMINO ACID AND PEPTIDE METABOLISM AS INFLUENCED BY GROSS GENE REARRANGEMENTS IN *DROSOPHILA PSEUDOOBSCURA*

by

Albert T. [Temple] Ellis

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF BIOLOGICAL SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1968

THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by Albert T. Ellis

entitled Amino Acid and Peptide Metabolism as Influenced by Gross Gene Rearrangements in Drosophila pseudoobscura.

be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

R M Harris — Dissertation Director

Dec. 28, 1967 — Date

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

R M Harris	Dec. 28, 1967
Willard Van Asdall	28 Dec 1967
James W. Berry	28 Dec. 1967
Robert L. Mellon	Dec. 28, 67
W. Terras	28 Dec. '67

*This approval and acceptance is contingent on the candidate's adequate performance and defense of this dissertation at the final oral examination. The inclusion of this sheet bound into the library copy of the dissertation is evidence of satisfactory performance at the final examination.

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Albert T. Ellis

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. Theodosius Dobzhansky for supplying the three Drosophila stocks used in this study and to Drs. Kaoru Matsuda and Stanley Alcorn for their assistance with many of the experimental techniques.

The author also wishes to extend his thanks to Dr. Robert M. Harris for his constant help, guidance and encouragement throughout the performance of these experiments.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ABSTRACT

AMINO ACID AND PEPTIDE METABOLISM AS INFLUENCED BY GROSS GENE REARRANGEMENTS IN DROSOPHILA PSEUDOOBSCURA

by

Albert T. Ellis

Amino acid and peptide metabolism in isogenic strains of Standard, Arrowhead and Chiricahua inversion races of Drosophila pseudoobscura have been studied by means of thin layer chromatography and electrophoresis. Both qualitative and quantitative differences among the zygote, 72 hour old larvae, 24 hour old pupae, 72 hour old pupae and 24 hour old imagoes of the ST/ST, AR/AR and CH/CH inversion homozygotes have been detected at both 16° C and 25° C. Quantitatively those metamorphic stages raised at 16° C generally contained more extractable free amino acids than those individuals raised at 25° C.

In the ST/ST and CH/CH inversion homozygotes raised at both 16° C and 25° C the number of extractable amino acids increases from the zygote stage through the 24 hour pupal stage followed by a decrease in the number of amino acids through the 72 hour pupal stage and then an increase in the number of amino acids in the 24 hour imago stage.

In the AR/AR inversion homozygote this general pattern was slightly altered. A decrease in the number of extractable amino acids occurred between the 72 hour pupal and 24 hour imago stage. The quantitative amino acid fluctuations occurring among the various metamorphic stages of the inversion homozygotes were not generally found among the same metamorphic stages of the inversion heterozygotes raised at both 16° C and 25° C. A quantitative pattern similar to that found in the inversion homozygotes was found only in the ST/CH inversion heterozygote raised at 25° C.

Numerous qualitative amino acid differences were also found. Of the twenty amino acids commonly occurring in proteins only alanine and tyrosine were not detected in any metamorphic stage of any genotype at either 16° C or 25° C. The remaining eighteen commonly occurring amino acids occurred in three general categories. Four amino acids were found in all metamorphic stages of certain genotypes of both inversion homozygotes and inversion heterozygotes. Many amino acids were restricted to metamorphic stages in either inversion homozygotes or inversion heterozygotes, however, no pattern as to genotype, metamorphic stage or temperature was evident.

Thirty distinctly resolved proteins were consistently observed in a 50% ammonium sulfate fraction obtained from

24 hour old inversion homozygote imagoes raised at both 16° C and 25° C. Eleven proteins were observed in the ST/ST genotype, eleven in the CH/CH genotype and eight in the AR/AR genotype. Two proteins were distinctly common to all three inversion homozygotes, one additional protein occurred in both the CH/CH and AR/AR genotypes while yet another protein was found in both the ST/ST and AR/AR inversion homozygotes at both 16° C and 25° C. Thirteen of the thirty proteins were found occurring only once among the three inversion homozygotes.

Thirty-seven distinct proteins were consistently observed in the inversion heterozygotes raised at both 16° C and 25° C. Fourteen proteins were found in the ST/CH hybrid, twelve in the ST/AR hybrid and eleven in the CH/AR hybrid. A number of proteins common to the various hybrids were observed. Evidence is presented which indicates that many of these proteins may represent hybridization of electrophoretic variants.

INTRODUCTION

The genetic control of physiological processes has become well established in biological literature during the past twenty-six years. The elegant work of Beadle (1946), Beadle and Tatum (1941), Horowitz (1950) and a host of other investigators with Neurospora crassa established the role of the gene in the enzymatic control of the individual steps in biochemical reactions. The structure and specificity of each polypeptide or enzyme is determined by a different gene (Horowitz, 1948). Studies by Ingram (1956) and Hunt and Ingram (1960) on human hemoglobin and recent studies on tryptophan synthetase from Escherichia coli by Yanofsky, Helinski and Maling (1961), Henning and Yanofsky (1963) and Yanofsky et al (1964) established the role of the gene in determining the primary structure of proteins. Studies by Jacob and Monod (1961) have further strengthened the gene-protein relationship by the discovery of genetic mechanisms for the regulation of protein synthesis.

Hubby (1963) and Hubby and Throckmorton (1965) have utilized genetically controlled protein structure to study the evolutionary relationships in the virilis group of Drosophila and protein differences in Drosophila melanogaster.

The most thorough understanding of polymorphism in sexual species has been obtained through studies of the inverted sections of chromosomes in various species of Drosophila. The first inversions were detected in Drosophila melanogaster through supression of crossover gametes in inversion heterozygotes (Sturtevant, 1926, 1931). The study of inversions was facilitated by the technique of observing the giant chromosomes in the salivary glands of the larval stage (Painter, 1934).

The objective of the present investigation involving inversion races of Drosophila pseudoobscura is to study the effect of large gene rearrangements on amino acid and peptide metabolism. The three principle inversion races utilized in this study offer an opportunity to analyze the effects of gross chromosome changes on the physiology of an organism. No studies of this nature appear in the literature although Dobzhansky and other investigators have described the effects of these inversions on the adaptability of these inversion races to various environmental conditions.

Dobzhansky and Sturtevant (1938) and Dobzhansky and Epling (1944) have shown that most natural populations of two closely related species, Drosophila pseudoobscura and Drosophila persimilis are mixtures of individuals with different gene arrangements in their third chromosomes.

Sixteen different gene arrangements are known in Drosophila pseudoobscura and eleven are known in Drosophila persimilis. Only one arrangement termed standard is common to both species. All of these arrangements are interrelated as overlapping inversions. Although a complete collection of these inversions is not found in any natural population, in some localities up to eight inversions occur together and both inversion homozygotes and inversion heterozygotes are found, giving rise to polymorphism. Chromosomal polymorphism due to inversions has been found in natural populations of about thirty species of Drosophila. Of the species examined in detail only D. virilis and D. hydei do not possess chromosomal polymorphism (Warters, 1944).

Studies with experimental populations maintained in population cages have shown that a stable equilibrium will be established under the experimental conditions with the inversion races present in the gene pool in definite frequencies. The establishment of stable equilibria in experimental populations is the rule whenever the competing inversion races are from the same geographical location, (Dobzhansky, 1947a, 1947b; Dobzhansky and Wallace, 1948). A similar situation has been found by Speiss (1950) in D. persimilis and by Levitan (1951) in D. robusta. The adaptive values of the inversion races are extremely sensitive to environmental conditions. Population experiments containing Standard and

Chiricahua inversion races maintained at 25° C exhibit a change in inversion frequencies and establishment of a stable equilibrium regardless of the initial frequencies of the inversion types. If these inversion types are raised at 16° C, however, no change in the relative frequencies is observed and the adaptive values of the homozygotes and heterozygotes are similar (Wright and Dobzhansky, 1946). Cyclic seasonal changes in the frequencies of certain inversion types take place in natural populations of some localities of Mt. San Jacinto in California (Dobzhansky, 1943). Dubinin and Tiniakov (1945, 1946b and 1946c) have observed cyclic seasonal changes in the frequencies of chromosome types in D. funebris. Small seasonal changes in the frequency of chromosomal inversions have also been found in populations of D. robusta (Carson and Stalker, 1949).

The inverted sections of the third chromosome of D. pseudoobscura may produce their adaptive advantage by modifying the physiology of the carriers either by position effect or by possessing mutant genes which differ from those in the chromosomes with the alternate inversion types. The effect may also be produced by a combination of both. Dobzhansky (1950c) has been able to show by means of experimental populations that when inversions from different geographical locations are made to compete with one another

the inversion heterozygote shows an adaptive advantage in only a very few cases. The adaptive value of the heterozygote is, therefore, not determined by the inversion alone but at least in part by the genes located within the inversions.

Heuts (1948) has been able to correlate certain physiological properties (temperature and humidity requirements) in D. pseudoobscura with specific inversion types. Evidence linking the inversion types with specific metabolic functions is, however, lacking in the literature.

There is considerable literature, however, correlating point mutations and specific metabolic patterns in other species of Drosophila. Nakamara, et al (1953) have studied amino acid metabolism in the four metamorphic stages of zygote maturation in lethal strains of an attached X strain of D. melanogaster. Nakamara, Imaizumi, and Takanami (1952) have shown that valine, isoleucine and lysine are stable quantitatively while the concentration of glutamic acid decreases during the early development of the zygote of D. virilis. During the embryological development of D. melanogaster the total ninhydrin positive material reaches a minimum shortly before hatching and then increases (Van der Crone-Gloor, 1959). Nakamara, Imaizumi and Kitazume (1951a) and Benz (1955) also have been able to show by means of paper chromatography that the concentration of

amino acids and peptides changes during the development of D. melanogaster. Castiglioni (1953) has chromatographed for fluorescent substances in different eye pigment mutants of D. melanogaster and found that there is a close correlation between the amount of pigment granules in histological sections and the intensity of fluorescence. The heterozygotes were intermediate between the homozygotes from which they were derived. Tondo and Cordeiro (1956) suggest that the electrophoretic properties of components of the red eye pigment in D. willistoni may be under genetic control as these six components differ only in their physico-chemical properties. Danneel and Zimmerman (1954) have shown by paper chromatographic analysis of pupae and imagoes that tryptophan metabolism in vermilion eye color mutants varies with the genotype. Hadorn and Mitchell (1950) also working with eye pigment mutants have shown differences in amino acid metabolism and fluorescent compounds during the metamorphic stages of D. melanogaster.

Beckman and Johnson (1963) have studied variations of leucine aminopeptidase (LAP) in D. melanogaster. Using pupal homogenates, these investigators have demonstrated the presence of two electrophoretic variants from different stocks. The F_1 hybrids resulting from a cross between these two strains showed the presence of both variants. Beckman and Johnson (1963) also were able to demonstrate

the presence of genetic variants of phosphatase in larval homogenates of different D. melanogaster stocks. Mixtures of homogenates of these different stocks contained both phosphatase variants.

Kikkawa (1963) has demonstrated the presence of fast and slow moving amylase components in D. virilis. Zymograms of F_1 hybrids between D. virilis containing the slow moving component and D. novamexicana containing the fast moving component show both the slow and fast moving components, indicating that each amylase gene produces its own product. Kikkawa (1965) has also demonstrated the presence of fast and slow moving amylase components in D. similans. These two components are controlled by allelic and co-dominant genes on chromosome number two. Doane (1966) has also studied α-amylase components by means of disc electrophoresis and has been able to demonstrate the presence of eight different banding patterns in various strains of D. melanogaster. Heterozygotes produced from these various strains show additive effects of the alleles from each parent indicating that no hybrid enzymes are produced and that the amylases are monomers.

MATERIALS AND METHODS

Stocks of the three inversion races of D. pseudo-obscura, Standard, Arrowhead and Chiricahua, were obtained from the laboratory of Dr. Theodosius Dobzhansky. Dr. Dobzhansky obtained these three stocks from the same geographical location, Pinion Flats, Mt. San Jacinto, California, and maintained them in the laboratory for many generations. As far as can be determined the strains are isogenic.

EXPERIMENTAL POPULATIONS

Establishment and Screening of Populations

Experimental populations of each of the three inversion races were established by mating virgin females from each of the inversion races to males from the same inversion race. The descendents of these matings were used in all subsequent experiments. These populations were carefully screened for ten generations to ascertain whether or not any morphological mutations were present. No detectable morphological mutations were observed during this initial screening period.

Following the initial screening, each succeeding generation was screened at two day intervals for the presence of mutations. Populations containing suspect individuals were discarded.

The purity of each inversion race was acertained by periodic random sampling of each generation through third instar larva salivary gland smears.

Inversion heterozygotes were produced by mating three females and two males from the various races. Only the F_1 individuals were utilized in the various experiments.

Growth Conditions

All populations were grown on cornmeal-molasses-agar medium (Strickberger, 1962) in 8 ounce flint glass bottles plugged with gauze wrapped cotton stoppers. Identical populations were maintained in Precision BOD temperature control chambers at both 16° C $\pm$ 0.5° C and 25° C $\pm$ 0.5° C. Stock populations raised at 25° C were transferred to new medium at four week intervals while stock populations raised at 16° C were transferred to new medium at six week intervals. Populations needed for the various experimental procedures were started whenever they were required. BOD boxes and all work surfaces were treated weekly with a 1:1 mixture of benzyl benzoate and mineral oil to prevent infestation with mites.

AMINO ACID ANALYSIS

Qualitative analysis of the free amino acids in the various metamorphic stages of both inversion homozygotes and inversion heterozygotes was accomplished by extracting the free amino acids then separating and identifying them by means of thin layer chromatography.

A series of experiments were performed to determine the number of individuals of each metamorphic stage necessary to produce the maximum number of ninhydrin positive spots. The data from Table 1 indicate that 200 zygotes, 100 seventy-two hour larva, 100 twenty-four hour pupae, 100 seventy-two hour pupae and 100 twenty-four hour imagoes produced the maximum number of free amino acids under the experimental conditions. These numbers of individuals were then used in all amino acid extractions.

Extraction Procedures

The appropriate number of individuals of each metamorphic stage was ground with a mortar and pestle in 20 ml of 80% ethanol (Smith, 1960). The macerated fly material in the 80% ethanol was then filtered and the mortar, pestle and filter paper were washed with an additional 10 ml of 80% ethanol. The grindate was discarded and three volumes (90 ml) of chloroform were added to the filtrate. This chloroform-ethanol mixture was shaken vigorously, then set aside to allow the two immiscible liquids to separate. Following

separation the aqueous layer was removed and evaporated to 0.5 ml to concentrate the amino acids and then spotted on thin layer plates.

Chromatographic Techniques

A slurry of 30 grams of Silica Gel G (Research Specialties Corp.) in 60 ml of distilled de-ionized water was prepared and spread by means of a chromatofilm spreader on 20 by 20 cm double strength glass plates. These thin layer plates were allowed to air dry for thirty minutes and were then stored in storage racks until needed. Prior to use the prepared plates were activated by heating in an oven at 105° C for thirty minutes (Truter, 1963). The plates were then allowed to cool and were used within four hours after activation.

The concentrated extracts were spotted on the thin layer plates with a 1.0 mm diameter nichrome wire loop. A series of experiments were run to determine the amount of extract necessary to produce the maximum number of spots on the thin layer plates (Table 2). For all experiments six drops of extract or standard were spotted on the thin layer material. Quantities of extract less than this amount resulted in fewer amino acids being detected while more than six drops of extract resulted in fewer amino acids being detected due to tailing.

With each extract analysis a series of twenty amino acid standards (Table 3) were also chromatographed as a control. These standard amino acid mixtures were prepared by dissolving 2 mg of amino acid (California Biochemical Corp.) per ml of distilled de-ionized water with 0.1 ml of iso-propanol added to each 50 ml of stock solution.

The chromatograms were developed by an ascending, one-dimensional technique in a solvent system composed of two parts iso-propanol to one part 17% ammonia.

Detection was accomplished by spraying the plates with 0.25% ninhydrin (California Biochemical Corp.) in n-butanol followed by oven drying at 60° C for fifteen minutes. The plates were also examined by ultraviolet for the presence of fluourescing amino acids. The minimum concentration of each amino acid standard detectable with this chromatographic technique was determined by chromatographing a series of dilutions of each amino acid standard. The minimum detectable concentrations varied but fell within a range of 0.001μg to 0.1μg (Table 4).

PROTEIN DIFFERENCES

Protein differences among the inversion homozygotes and inversion heterozygotes were demonstrated by extraction of the proteins from twenty-four hour old adult flies and separation of the proteins by zone electrophoresis.

Extraction

Inversion homozygotes and inversion heterozygotes were grown on cornmeal-molasses-agar medium (Strickberger, 1962) at both 16° C ± 0.5° C and 25° C ± 0.5° C. Adult flies were collected twenty-four hours after eclosion and stored in glass vials at -20° C.

The extraction method employed was a modification of the technique used by Hubby (1963) in his comparison of proteins in various species of Drosophila.

All glassware, solutions and equipment used in the extractions were equilibrated at 4° C for a period of twenty-four hours. For each extraction, 6 g of flies were suspended in 30 ml of a 0.1 M Tris-HCl buffer at pH 7.0 and thoroughly ground with a mortar and pestle. The slurry was then centrifuged in a Serval Model SS-I centrifuge at a relative centrifugal force of 12,000 x gravity for a period of 20 minutes. The pellet was discarded and the supernatant was taken to 50% saturation with ammonium sulfate (calculated at 0° C). The solution was allowed to stand for 30 minutes and then centrifuged at a relative centrifugal force of 24,000 x gravity for 30 minutes. Both centrifugation and equilibration were carried out at 0° C. The supernatant was discarded and the centrifugate suspended in 3 ml of distilled de-ionized water and dialyzed for 24 hours against 4 l of distilled de-ionized water

which was stirred gently with a magnetic stirrer. The dialyzed material was then lyophilyzed and stored as a dry powder at -20° C.

From the starting material at 6.0 g wet weight an average yield of 50 mgs of lyophilyzed material was obtained from the 50% ammonium sulfate precipitation (Table 5).

Electrophoresis

Electrophoresis was performed horizontally in plexiglass trays 50 mm wide by 150 mm long by 6 mm deep (Smith, 1960) laid on a model E6000-2D electrophoresis cell (Research Specialties Co.).

Starch gels were prepared by a modification of the technique outlined by Smithies (1955). Fifteen grams of starch for electrophoresis (Mann Research Laboratories, Inc.) were dissolved in 100 ml of Tris-borate buffer containing 1.5 micromoles of EDTA at a pH of 8.9. This buffer was also used for the electric bridge. The gel was poured into the plexiglass trays, cooled, trimmed and used within four hours.

Samples consisted of a 5% solution of the lyophilyzed materials dissolved in the Tris-borate-EDTA buffer. Twenty microliters of the sample were absorbed onto 5 by 20 mm pieces of Whatman 3MM filter paper which were then inserted into slits of the same size out into the starch gel. After insertion of the samples a potential difference of 400 volts

was maintained across the gel for 90 minutes. The power source used in electrophoresis of the extracts from the inversion homozygotes was a Model HV 5000-3 High Voltage Power Supply obtained from Servonuclear Corporation while the power source for the studies with the inversion heterozygotes was a Braun power supply number 27391-8 (Van Waters and Rogers, Inc.).

The 6.0 mm thick blocks of starch gel were sliced horozontally into four 1.5 mm thick slices. The top and bottom slices generally gave erratic results and were routinely discarded. The two central slices were removed to staining trays and stained in a solution of 0.1 g of Acid Black in 20% acetic acid. Staining was completed after 30 minutes. The excess dye was removed by washing the gel in a continuous stream of 20% acetic acid for 8 hours. Diagrams were then made of the bands present in the starch gel.

RESULTS

AMINO ACIDS

Analysis of the free amino acids in the various metamorphic stages of both inversion homozygotes and inversion heterozygotes revealed a number of qualitative and quantitative differences in flies raised at both 16° C and 25° C.

Quantitative Differences

The number of free amino acids extracted from the various metamorphic stages of both inversion homozygotes and inversion heterozygotes tended to decrease when the individuals were raised at 25° C. In general those individuals raised at 16° C contained more free amino acids than individuals raised at 25° C (Tables 6, 7). Exceptions to this general pattern were found, however. In three different metamorphic stages the same number of free amino acids were extracted from individuals raised at 16° C and at 25° C. Four amino acids were extracted from the zygote stage of the ST/CH hybrid at both 16° C and 25° C while six amino acids were obtained from the 24 hour pupa stage of the CH/AR hybrid at both 16° C and 25° C (Table 7). Among the CH/CH inversion homozygotes, seven amino acids were obtained from the 24 hour imagoes raised at both

16° C and 25° C (Table 6).

In four different metamorphic stages the number of amino acids obtained from individuals raised at 25° C exceeded the number of amino acids extracted from individuals raised at 16° C. Among the inversion heterozygotes this situation was found in the zygote stage of the CH/AR hybrid and in the 24 hour pupal stage of the ST/CH hybrid (Table 7). Among the inversion homozygotes an increased number of amino acids were found at 25° C in the 24 hour imago stage of the ST/ST genotype and in the 24 hour imago stage of the AR/AR genotype (Table 6).

A general pattern as to numbers of amino acids obtained from the various metamorphic stages of the inversion homozygotes is evident at both 16° C and 25° C. In the ST/ST and CH/CH genotypes the number of amino acids increases from the zygote stage through the 24 hour pupal stage followed by a decrease in the 72 hour pupal stage, then an increase in the 24 hour imagoes (Table 6). This pattern was modified slightly in the AR/AR genotype. At 16° C there was a decrease in numbers of amino acids from the 72 hour pupa to the 24 hour imagoes. At 25° C, five amino acids were found in the zygote and 24 hour pupa and 72 hour pupa stages and seven in the 24 hour imago stage (Table 6).

This pattern of amino acid fluctuation found in the inversion homozygotes was found only in the ST/CH hybrid individuals raised at 25° C. In the ST/CH hybrids raised at both 16° C and 25° C the pattern was altered with no general pattern being evident (Table 7).

Qualitative Differences

The data indicate a variety of qualitative differences were also found. Of the twenty amino acids commonly found in proteins, alanine and tyrosine did not appear in any metamorphic stage of any genotype at either 16° C or 25° C (Table 8).

Amino Acids Found in all Metamorphic Stages of Specific Genotypes. Four amino acids were found in all metamorphic stages of certain genotypes of both inversion homozygotes and inversion heterozygotes. Among the inversion homozygotes, threonine was found in all stages of the AR/AR, ST/CH and ST/AR genotypes raised at 16° C (Table 8; Appendix A, Tables 1, 3, 5, 7, 9, 11, 13, 15, 17, 19) and histidine occurred in all stages of the CH/CH genotype raised at 16° C (Table 8; Appendix A, Tables 1, 3, 5, 7, 9). Arginine was found in all stages of the ST/CH hybrid at both 16° C and 25° C (Table 8; Appendix A, Tables 11, 12, 13, 14, 15, 16, 17, 18, 19, 20). Cysteine occurred in all stages of two inversion heterozygotes, ST/AR and CH/AR raised at 16° C (Table 8; Appendix A, Tables 11, 13, 15, 17, 19).

Amino Acids Found only in Metamorphic Stages of Inversion Homozygotes. Many amino acids were restricted to the inversion homozygotes although no pattern as to genotype, metamorphic stage or temperature was found.

Phenylalanine appeared only in the 24 hour pupa of the CH/CH genotype at both 16° C and 25° C and in the 24 hour pupa of the AR/AR genotype raised at 16° C (Table 8; Appendix A, Tables 5, 6). Tryptophan occurred only in the 24 hour pupa stage of the ST/ST, AR/AR and CH/CH genotypes raised at 16° C (Table 8; Appendix A, Table 5), in the 24 hour pupa stage of the ST/ST genotype raised at 25° C (Table 8; Appendix A, Table 6) and in the zygote stage of the CH/CH genotype raised at both 16° C and 25° C (Table 8; Appendix A, Tables 1, 2). Methionine was restricted to the 72 hour pupa stage of the ST/ST and AR/AR genotypes raised at both 16° C and 25° C (Table 8; Appendix A, Tables 7, 8). Asparagine was found in the zygote stage of the ST/ST and AR/AR genotypes raised at both 16° C and 25° C (Table 8; Appendix A, Tables 1, 2), the CH/CH genotype raised at 16° C (Table 8; Appendix A, Table 1) as well as the 24 hour imago stage of the CH/CH genotype raised at 25° C (Table 8; Appendix A, Table 10). Proline occurred in the 24 hour pupa stage of the CH/CH genotype raised at 16° C (Table 8; Appendix A, Table 5) and in the 24 hour pupa stage of the AR/AR genotype raised at 25° C

(Table 8; Appendix A, Table 6). Serine was restricted to both the 72 hour pupa and 72 hour larva of the ST/ST genotype raised at 16° C (Table 8; Appendix A, Tables 3, 7), the 72 hour larva and pupal stages of the CH/CH genotype raised at both 16° C and 25°C (Table 8; Appendix A, Tables 3, 4, 7, 8) and to the 72 hour larva and pupal stages of the AR/AR genotype raised at 16° C (Table 8; Appendix A, Tables 3, 7) as well as the 72 hour pupal stage raised at 25° C (Table 8; Appendix A, Table 8). Glutamic acid occurred in the 24 hour imago stage of all inversion homozygotes at both 16° C and 25° C (Table 8; Appendix A, Tables 9, 10).

Amino Acids Found only in Metamorphic Stages of Inversion Heterozygotes. A number of amino acids were found only in certain stages of the inversion heterozygotes. As with the inversion homozygotes, no specific pattern was evident from the data.

Glycine was found in the 24 hour imago stage of only the ST/CH hybrid raised at 25° C (Table 8; Appendix A, Table 20). Isoleucine occurred in the zygote and 24 hour imago stages of the ST/CH genotype at both 16° C and 25° C (Table 8; Appendix A, Tables 11, 12, 19, 20), in the zygote and 24 hour imago stages of the ST/AR genotype at 16° C (Table 8; Appendix A, Tables 11, 19). Serine was found in the zygote stage only of the ST/AR genotype at both 16° C

and 25° C (Table 8; Appendix A, Tables 11, 12). Aspartic acid occurred in the 72 hour and 24 hour pupa of all the inversion heterozygotes. In the ST/CH hybrid it was found in the 72 hour pupa raised at 16° C (Table 8; Appendix A, Table 17) and in the 24 hour pupa raised at both 16° C and 25° C (Table 8; Appendix A, Tables 15, 16), in the ST/AR hybrid in the 72 hour pupa raised at both 16° C and 25° C (Table 8; Appendix A, Tables 17, 18) as well as the 24 hour pupa raised at 16° C (Table 8; Appendix A, Tables 15, 16) and in the CH/AR hybrid in both the 72 and 24 hour pupae raised at 25° C (Table 8; Appendix A, Tables 16, 18). In the ST/CH hybrid glutamic acid was found in the zygote and 72 hour pupa at 16° C (Table 8; Appendix A, Tables 11, 17) and the 24 hour pupa raised at 25° C (Table 8; Appendix A, Table 16). In the ST/AR hybrid it was found in the zygote, 24 hour pupa and 72 hour pupa raised at 16° C (Table 8; Appendix A, Tables 11, 15, 17) while it was found in the zygote only at 25° C (Table 8; Appendix A, Table 12). Glutamic acid was also restricted to the zygote stage of the CH/AR hybrid raised at 25° C (Table 8; Appendix A, Table 11). Lysine was restricted to the 72 hour larva in the ST/CH hybrid at 16° C (Table 8; Appendix A, Table 13), the ST/AR hybrid at 25° C (Table 8; Appendix A, Table 14) and the CH/AR hybrid at both 16° C and 25° C (Table 8; Appendix A, Tables 13, 14). Lysine also occurred

in the 24 hour imagoes of all inversion heterozygotes at both 16° C and 25° C (Table 8; Appendix A, Tables 19, 20). Arginine occurred in the zygote stage only of the ST/CH hybrid at 16° C and 25° C (Table 8; Appendix A, Tables 11, 12), the ST/AR hybrid at 16° C (Table 8; Appendix A, Table 11) and the CH/AR hybrid at 25° C (Table 8; Appendix A, Table 12). Glutamine was found in the 72 hour pupa stage of the ST/CH genotype raised at both 16° C and 25° C (Table 8; Appendix A, Tables 17, 18) and the ST/AR genotype raised at 16° C (Table 8; Appendix A, Table 17). Cysteine occurred in the 72 hour pupa of the ST/CH hybrid raised at 25° C (Table 8; Appendix A, Table 18) in the 24 hour imagoes of the ST/AR hybrid raised at 16° C (Table 8; Appendix A, Table 19) and in the 72 hour pupa stage of the ST/AR genotype raised at both 16° C and 25° C (Table 8; Appendix A, Tables 17, 18). Cysteine also was found in the CH/AR hybrids in the 72 hour pupa raised at 16° C (Table 8; Appendix A, Table 17) and in the 72 hour pupa, 24 hour pupa and 24 hour imagoes raised at 25° C (Table 8; Appendix A, Tables 16, 18, 20).

PROTEIN ANALYSIS

In the 50% ammonium sulfate fraction studied, thirty distinctly resolved proteins were consistently observed in the inversion homozygotes at both 16° C and 25° C. Eleven of these proteins were observed in the

ST/ST genotype (Figs. 1, 2; Appendix B, Figs. 1, 4), eleven in the CH/CH genotype (Figs. 1, 2; Appendix B, Figs. 2, 5) and eight in the AR/AR genotype (Figs. 1, 2; Appendix B, Figs. 3, 6). Thirty-seven distinctly resolved proteins were consistently observed in the inversion heterozygotes at both 16° C and 25° C. Fourteen proteins were found in the ST/CH hybrid (Figs. 3, 4; Appendix B, Figs. 7, 10), twelve in the ST/AR hybrid (Figs. 3, 4; Appendix B, Figs. 8, 11) and eleven were found in the CH/AR hybrid (Figs. 3, 4; Appendix B, Figs. 9, 12).

Inversion Homozygotes

In the inversion homozygotes a number of proteins common to the three inversion races were found. Intensely dark staining bands 7 and 8 were common to all three genotypes at both 16° C and 25° C (Figs. 1, 2; Appendix B, Figs. 1, 2, 3, 4, 5, 6). Band 3 was found only in the ST/ST and AR/AR races (Figs. 1, 2; Appendix B, Figs. 1, 3, 4, 6) at both 16° C and 25° C. In the AR/AR genotype band 3 occurred as a very faintly staining band. Band 9 occurred in the CH/CH and AR/AR races at both 16° C and 25° C (Figs. 1, 2; Appendix B, Figs. 2, 3, 5, 6) as a light staining band. Band 20 was found in the ST/ST race as a light staining band at both 16° C and 25° C (Figs. 1, 2; Appendix B, Figs. 1, 4) and as a dark staining wide band in the CH/CH race at both 16° C and 25° C (Figs. 1, 2; Appendix

B, Figs. 2, 5). Band 22 was found as a dark staining band in the CH/CH race at both 16° C and 25° C (Figs. 1, 2; Appendix B, Figs. 2, 5) and as a light staining band in the AR/AR race at both 16° C and 25° C (Figs. 1, 2; Appendix B, Figs. 3, 6).

Thirteen of the thirty bands were found occurring only once among the three inversion homozygotes. Bands 2, 5, 14, 17 and 19 were found only in the ST/ST genotype at both 16° C and 25° C (Figs. 1, 2; Appendix B, Figs. 1, 4). Bands 1, 4, 6, 12, 16 and 18 occurred only in the CH/CH genotype at both 16° C and 25° C (Figs. 1, 2; Appendix B, Figs. 2, 5). Bands 10, 15 and 21 were found only in the AR/AR race at both 16° C and 25° C (Figs. 1, 2; Appendix B, Figs. 3, 6).

Inversion Heterozygotes

In the inversion heterozygotes a large number of distinct bands were consistantly observed to be common to all three hybrids. Bands 7, 8, 10, 17 and 20 occurred as dark staining bands while bands 9 and 19 occurred as faint bands at both 16° C and 25° C (Figs. 3, 4; Appendix B, Figs. 7, 8, 9, 10, 11, 12). Band 1 was found as a dark staining band and band 6 was found as a light staining band in the ST/CH and CH/AR hybrids at both 16° C and 25° C (Figs. 3, 4; Appendix B, Figs. 7, 9, 10, 12). Band 22 appeared as a wide, dark staining band in the ST/CH

and CH/AR hybrids while it was found in the ST/AR hybrid as a narrow, light staining band (Figs. 3, 4; Appendix B, Figs. 7, 8, 9, 10, 11, 12). Band 3 occurred as a light staining band in the ST/CH and ST/AR hybrids at both 16° C and 25° C (Figs. 3, 4; Appendix B, Figs. 7, 8, 10, 11). Band 12 was found as a dark staining band only in the CH/AR hybrid at both 16° C and 25° C (Figs. 3, 4; Appendix B, Figs. 9, 12). Bands 5, 13 and 14 appeared as wide, dark staining bands in the ST/CH and ST/AR hybrids at both 16° C and 25° C (Figs. 3, 4; Appendix B, Figs. 7, 8, 10, 11).

Table 1.

Number of individuals of each metamorphic stage of D. pseudoobscura used in amino acid extractions. Numbers refer to number of distinct ninhydrin positive spots. Standard race used as a basis.

Number of Individuals

Metamorphic Stage	50		100		150		200		250	
	16°C	25°C	16°C	25°C	16°C	25°C	16°C	25°C	16°C	25°C
Zygote	1	1	2	1	3	3	4	3	3	3
72 Hour Larvae	4	4	7	5	7	4	6	4	5	4
24 Hour Pupae	8	7	9	7	8	7	7	6	7	6
72 Hour Pupae	5	4	6	5	5	5	5	4	4	4
24 Hour Imagoes	4	4	6	7	5	5	5	4	4	4

Table 2.

Number of drops of amino acid extracts spotted on thin layer chromatographic plates. Standard race used as a basis. Numbers refer to the number of distinct ninhydrin positive spots present on the plates after development and detection.

Number of Drops Spotted

Metamorphic Stage	2		4		6		8		10	
	16°C	25°C	16°C	25°C	16°C	25°C	16°C	25°C	16°C	25°C
Zygote	2	2	3	2	4	3	3	2	2	1
72 Hour Larvae	3	4	6	5	7	5	6	5	6	4
24 Hour Pupae	2	4	5	6	9	7	8	7	8	6
72 Hour Pupae	2	5	5	5	6	5	6	4	5	5
24 Hour Imagoes	3	4	6	5	6	7	6	5	5	5

Table 3.

Amino Acid Standards

Alanine	Aspartic Acid
Glycine	Glutamic Acid
Valine	Lysine
Leucine	Arginine
Isoleucine	Histidine
Serine	Cysteine
Threonine	Methionine
Tyrosine	Asparagine
Phenylalanine	Glutamine
Tryptophan	Proline

Table 4.

Minimum concentrations in µg of standard amino acids detectable with ninhydrin using a one-dimensional technique on Silica Gel G.

Amino Acid	Conc.	Amino Acid	Conc.
Alanine	0.01	Aspartic Acid	0.1
Glycine	0.001	Glutamic Acid	0.04
Valine	0.01	Lysine	0.005
Leucine	0.01	Arginine	0.01
Isoleucine	0.01	Histidine	0.05
Serine	0.01	Cysteine	0.01
Threonine	0.05	Methionine	0.01
Tyrosine	0.03	Asparagine	0.1
Phenylalanine	0.05	Glutamine	0.04
Tryptophan	0.05	Proline	0.1

Table 5.

Yield in mg of lyophilyzed protein extract of inversion homozygotes and inversion heterozygotes of *D. pseudoobscura*.

Inversion Homozygotes			
Genotype	ST/ST	CH/CH	AR/AR
Weight	50 mgs	49 mgs	49 mgs

Inversion Heterozygotes			
Genotype	ST/CH	ST/AR	CH/AR
Weight	51 mgs	50 mgs	49 mgs

Table 6.

Number of different amino acids present in the various metamorphic stages of inversion homozygotes raised at 16° C and 25° C.

Metamorphic Stage	ST/ST		CH/CH		AR/AR	
	16°C	25°C	16°C	25°C	16°C	25°C
Zygote	4	3	5	3	6	5
72 Hour Larvae	7	5	8	6	7	5
24 Hour Pupae	9	7	11	7	8	6
72 Hour Pupae	6	5	6	5	7	6
24 Hour Imagoes	6	7	7	7	5	7

Table 7.

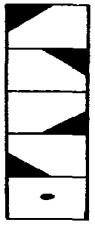
Number of different amino acids present in the various metamorphic stages of inversion heterozygotes raised at 16° C and 25° C.

Metamorphic Stage	ST/CH		ST/AR		CH/AR	
	16°C	25°C	16°C	25°C	16°C	25°C
Zygote	4	4	7	5	3	4
72 Hour Larvae	7	6	6	4	7	4
24 Hour Pupae	6	7	9	5	6	6
72 Hour Pupae	6	5	8	5	5	3
24 Hour Imagoes	9	8	8	5	9	6

Table 8. Amino acids present in the various metamorphic stages of inversion homozygotes and inversion heterozygotes raised at 16° C and 25° C.

Genotype	ST/ST		CH/CH		AR/AR		ST/CH		ST/AR		CH/AR	
Temp. ° C	16	25	16	25	16	25	16	25	16	25	16	25
Gylcine												
Alanine												
Valine												
Leucine												
Isoleucine												
Serine												
Threonine												
Tyrosine												
Phenylalanine												
Tryptophan												
Aspartic Acid												
Glutamic Acid												
Lysine												
Arginine												
Histidine												
Cysteine												
Methionine												
Asparagine												
Glutamine												
Proline												

KEY TO SYMBOLS



Zygote

Seventy-two Hour Larvae

Twenty-four Hour Pupae

Seventy-two Hour Pupae

Twenty-four Hour Imagoes

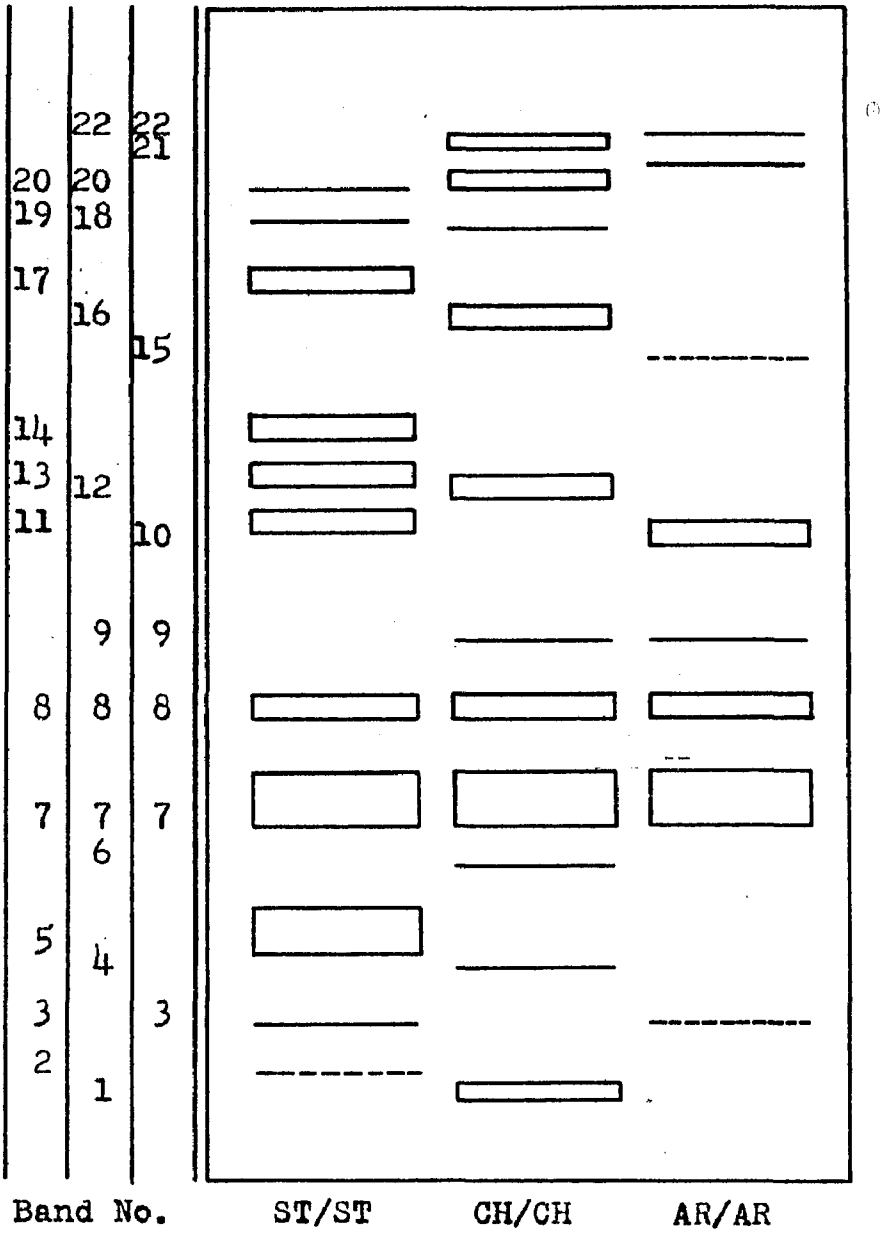


Fig. 1. Protein fractions from ST/ST, CH/CH and AR/AR inversion homozygotes raised at 16° C.

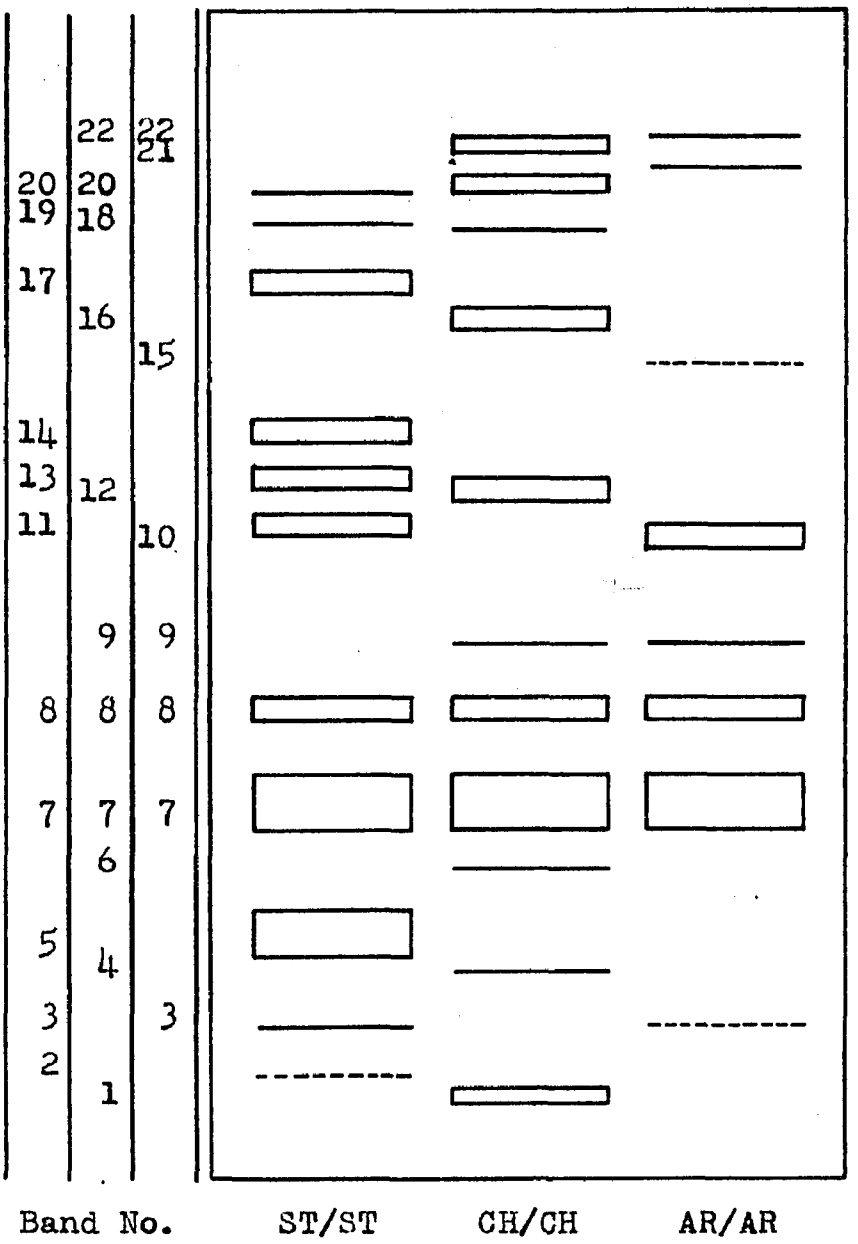


Fig. 2. Protein fractions from ST/ST, CH/CH and AR/AR inversion homozygotes raised at 25° C.

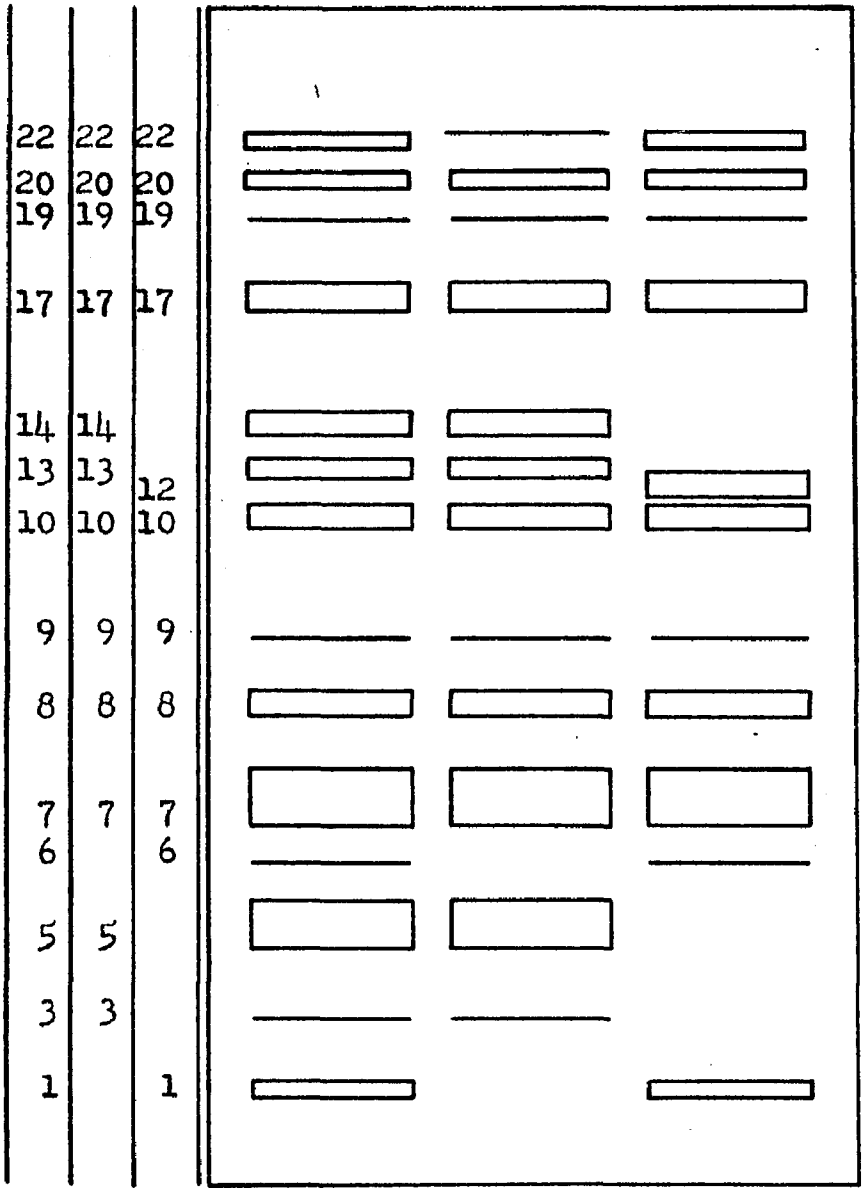


Fig. 3. Protein fractions from ST/CH, ST/AR and CH/AR inversion heterozygotes raised at 16° C.

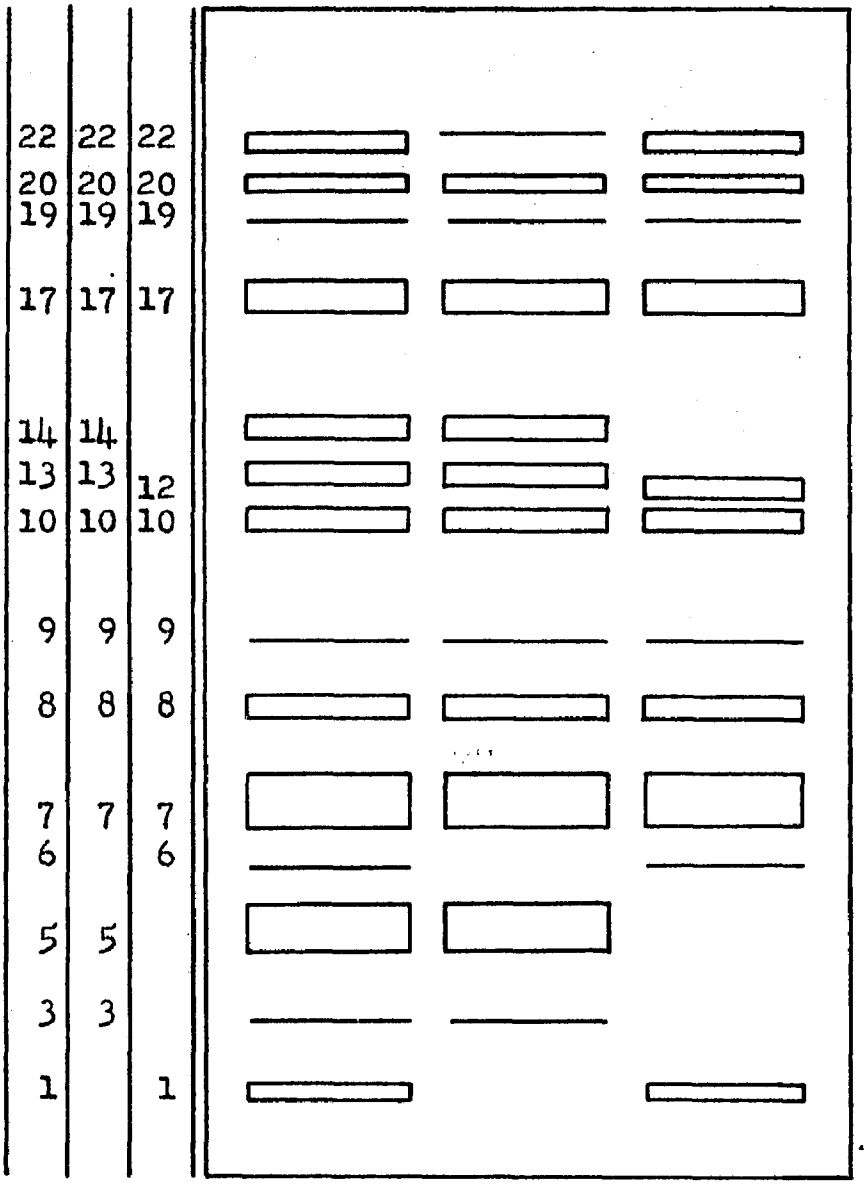


Fig. 4. Protein fractions from ST/CH, ST/AR and CH/AR inversion heterozygotes raised at 25° C.

DISCUSSION

In recent years the colinearity of the amino acid sequence in proteins with the nucleotide sequence in the gene has become well established in genetic literature (Yanofsky et al, 1964). A mutation in the genetic material could result in the production of a polypeptide in which at least one amino acid could be replaced, deleted or added to the polypeptide chain. In some cases, these amino acid substitutions, deletions or additions would result in a change in the net electrostatic charge on the polypeptide, which in turn would result in a change of the net charge on the protein (enzymatic or other protein) of which the polypeptide is a component. Proteins, as far as is known, are composed of polypeptides produced by one or more different structural genes.

Mutations in the genetic material may be manifested physiologically in at least two ways. Nakamara et al, (1953) Daneel and Zimmerman (1954), Hadorn and Mitchell (1950) and other investigators have demonstrated that genic alteration results in a demonstrable change in amino acid metabolism. Ingram (1956) and Hunt and Ingram (1960) and many other investigators have shown that

alteration of the primary structure of a protein resulting in a change in the net electrostatic charge produces proteins that possess different electrophoretic mobilities. Change in a single amino acid is sufficient to bring about differing mobilities.

In the past few years many naturally occurring polymorphic proteins (isozymes) have been demonstrated in a variety of organisms, among these are the esterases of Tetrahymena (Allen, 1960), maize (Schwartz, 1960) and mice (Popp, 1961). In most instances these polymorphic proteins have been detected by their different electrophoretic mobilities. Since 1956 at least six gene-enzyme systems have been identified in Drosophila by Forrest, Glassman and Mitchell (1956), Glassman and Mitchell (1959), Baglioni (1960), Lewis and Lewis (1961a, 1961b, 1962), Kikkawa (1960) and Wright (1961, 1963).

Hubby and Lewontin (1966) and Lewontin and Hubby (1966) have recently utilized protein polymorphism to study the extent of heterozygous loci in natural populations of D. pseudoobscura.

With one exception, no attempt has been made to utilize isogenic lines in these studies. Hubby and Lewontin have utilized strains of D. pseudoobscura that have been inbred since 1957 and probably represent homozygous lines. A number of these lines were homozygous for the Arrowhead inversion in the third chromsome.

The study being reported here is an attempt to determine the effect of the Standard, Arrowhead and Chiricahua inversions on otherwise identically homozygous strains of D. pseudoobscura.

The data obtained from the studies on both amino acid metabolism and electrophoretic mobilities of the proteins from the 50% ammonium sulfate solubility class indicate a possible effect from the inversion. Although no specific quantitative or qualitative pattern is evident from the amino acid studies, the differences in numbers and types of amino acids present in the various stages among the inversion homozygotes and inversion heterozygotes indicate that the inversions are possibly involved in the differing metabolic patterns. Although no specific qualitative or quantitative metabolic pattern is evident a temperature pattern is indicated from the data. The data in tables 6 and 7 indicate a general pattern in which those individuals raised at 16° C contain a greater number of extractable free amino acids than those individuals raised at 25° C. This alteration of amino acid metabolism in individuals raised at 16° C is possibly significant in view of the findings of Wright and Dobzhansky (1946) that no change in the relative frequencies of competing inversion types occurs in experimental populations raised at 16° C while shifts in inversion frequencies occur in experimental populations raised at 25° C. Temperature sensitive enzymes

involved in amino acid metabolism whose functioning is inhibited or completely stopped at lower temperatures would result in a greater number of free amino acids present in flies raised at the lower temperature. Incorporation of amino acids into required proteins would then be limited.

Further evidence for the involvement of the inversions in the metabolism of these three races is found in the data obtained from the analysis of the proteins in the 50% ammonium sulfate solubility class. Although essentially the same quantity of lyophilyzed material was obtained from the three inversion homozygotes and three inversion heterozygotes, these various fractions contained many proteins with different electrophoretic mobilities.

One of the principle genetic functions of inversions is maintaining the integrity of the block of genes located within the inversion through the supression of crossover gametes in the heterozygote (Dobzhansky, 1950c). The genes located within the different inversions may produce some proteins that are completely different in structure and function or they may produce proteins that are involved in the same function but differ in their physico-chemical properties through alteration of the protein structure. In the latter situation heterozygotes produced by crossing the various inversion homozygotes might possess both forms of the protein or in some cases they might also have hybrid

forms of the protein.

As would be expected with any solubility class, proteins common to all three inversion races were found (bands 7 and 8 in Figs. 1, 2; Appendix B, Figs. 1, 2, 3, 4, 5, 6). In addition to these common proteins the electrophoretic analysis of the 50% ammonium sulfate solubility class has revealed a number of proteins among the three inversion races that may be related as electrophoretic variants produced by different alleles within the inversions. Band 20 in the ST/ST and CH/CH inversion homozygotes (Figs. 1, 2; Appendix B, Figs. 1, 2, 3, 4, 5, 6) have essentially the same electrophoretic mobilities, however, the width and intensity of staining of the band from the CH/CH homozygote indicates a much greater concentration. Band 21 from the AR/AR homozygote (Figs. 1, 2; Appendix B, Figs. 3, 6) has a mobility greater than the mobility of band 20 from the ST/ST homozygote but it overlaps with the wide band 20 from the CH/CH homozygote (Figs. 1, 2; Appendix B, Figs. 1, 4, 2, 5). In the three inversion heterozygotes only a single wide band (band 20) occurs in this position indicating a possible hybridization (Figs. 3, 4; Appendix B, Figs. 7, 8, 9, 10, 11, 12). Hybridizations of this type have been described by Beckman and Johnson (1946) for larval alkaline phosphatase in D. melanogaster, by Wright (1963) for esterase 6 in D. melanogaster, by Hubby and Lewontin (1966) for esterase 5 in D. pseudoobscura and by Kikkawa (1960) for

amylase-Amy in D. melanogaster. This same situation could also account for band 22 in the CH/CH and AR/AR genotypes. This particular band occurs in the CH/CH genotype as a wide, dark staining band and in the AR/AR genotype as a narrow, light staining band and is absent in the ST/ST genotype. In the inversion heterozygote, band 22 appears as a wide, dark staining band in the ST/CH hybrid, a narrow, light staining band in the ST/AR hybrid and as a wide, dark staining band in the CH/AR hybrid. Band 18 in the CH/CH homozygote and band 19 in the ST/ST homozygote are possibly different forms of the same protein. In all three hybrids band 19 appears as a single, narrow, light staining band. The data also indicate that proteins produced by one or more of the inversion homozygotes are also produced when that particular homozygote is hybridized with another inversion homozygote in which the protein is not found. Band 22 found as a wide, dark staining band in the CH/CH homozygote and as a narrow, light staining band in the AR/AR homozygote is absent from the ST/ST homozygote (Figs. 1, 2; Appendix B, Figs. 1, 2, 3, 4, 5, 6). In the ST/CH and CH/AR hybrids, however, band 22 is present as a wide, dark staining band while it is present as a narrow, light staining band in the ST/AR hybrid (Figs. 3, 4; Appendix B, Figs. 7, 9, 10, 12). Band 14 found as a wide, dark staining band only in the ST/ST homozygote (Figs. 1, 2;

Appendix B, Figs. 1, 4) is found as a wide, dark staining band in all hybrids involving the Standard inversion (Figs. 3, 4; Appendix B, Figs. 7, 8, 10, 11). Band 6 found as a narrow, light staining band and band 1 found as a wide, dark staining band only in the CH/CH homozygote (Figs. 1, 2; Appendix B, Figs. 1, 5) are found in all hybrids involving the Chiricahua inversion (Figs. 3, 4; Appendix B, Figs. 7, 9, 10, 12). Band 9 found as a narrow light staining band in both the CH/CH and AR/AR homozygotes is also found as a narrow, light staining band in the ST/CH hybrid.

Dobzhansky (1950c) has conducted a series of experiments with artifical populations in which inverted third chromosomes with one gene arrangement from one geographical location were made to compete with chromosomes with a different gene arrangement from locations hundreds of miles away. Inversion heterozygotes with third chromosomes from widely separated geographical locations showed heterosis in only a very few cases. The adaptive value of the heterozygote is apparently not determined by the inversion alone but by genes located within the inversion.

The gene complexes within the various inversions found in the population of a specific geographical location, have through long periods of natural selection, become coadapted and the inversion heterozygotes possess

a high adaptive value. Inversions then become selective only if they arise in chromosomes in which adaptive gene complexes exist.

The inversion heterozygotes differ from the inversion homozygotes in one important respect. The inversion heterozygotes have within the same individuals proteins found in only one or the other of the inversion homozygotes and in some cases they possess a third form of the protein, the hybrid protein. Heteromorphy of protein structure would be selectively advantageous if the proteins were involved in critical metabolic functions and the proteins were affected by a particular selection factor. Dobzhansky (1947a, 1947b, 1948) has described altitudinal and seasonal changes in inversion frequencies in natural populations of D. pseudoobscura. Dobzhansky (1948) and Wright and Dobzhansky (1946) have also reported shifts in inversion frequencies under experimental conditions. In general the frequency of the Standard (ST) inversion decreases during the cool season from March to June while the frequency of the Chiricahua (CH) inversion increases. During the hot season, from June to August the opposite shifts occur with the frequency of the ST inversion increasing and the frequency of the CH inversion decreasing. Hypothetically the genes (allelic or non allelic) within the various inversions could produce enzymatic or other proteins whose optimum

rate of function occurs at different temperatures. Such temperature sensitive enzymes have been described in the Himalayan allele of the coat color multiple allelic series in rabbits (Wagner and Mitchell, 1955) and for tyrosinase in Neurospora crassa (Horowitz and Shen, 1952). The presence of different proteins affecting the same function or the presence of physico-chemical variations of the same functional protein could add to the flexibility of organisms that inhabit variable environments. Such a hypothesis is strengthened by high selective values of the heterozygous individuals afflicted with sickle-cell trait.

The data obtained from this series of experiments indicate that the inversion homozygotes produce some proteins that are characteristic of the specific homozygote. The data also indicate that many of these proteins either occur as hybrids or are found as distinct bands in heterozygous combinations. These heterozygotes were produced by crossing different inversion homozygotes, one of which did not produce the protein.

It is evident from the data that the electrophoretic pattern of proteins extracted from the inversion homozygotes raised at 16° C as well as the electrophoretic pattern of the proteins extracted from the inversion heterozygotes raised at 16° C did not differ from that of those individuals raised at 25° C. Evidence has been

presented, however, that indicates a difference in amino acid metabolism at 16° C and 25° C. While no evidence exists to explain the temperature effect differences that exist between these two approaches, it should be borne in mind that only one major solubility class of proteins was studied. Ammonium sulfate precipitation separates proteins into major solubility classes, while electrophoresis then separates the various proteins in these classes according to their electrophoretic mobilities. Further studies with other ammonium sulfate solubility classes might reveal temperature differences.

APPENDIX A

LIST OF TABLES

Table 1.

Inversion Homozygotes

Zygotes Raised at 16° C. Plus indicates presence of the amino acid.

Amino Acid	ST/ST	CH/CH	AR/AR
Asparagine	+	+	+
Cysteine			+
Gylcine	+	+	+
Histidine	+	+	+
Leucine	+	+	+
Threonine			+
Tryptophan		+	

Table 2.

Inversion Homozygotes

Zygotes raised at 25° C. Plus indicates presence of amino acid.

Amino Acid	ST/ST	CH/CH	AR/AR
Asparagine	+		+
Glycine	+		+
Histidine	+	+	
Leucine		+	+
Serine			+
Threonine			+
Tryptophan		+	

Table 3.

Inversion Homozygotes

Seventy-two hour larva raised at 16° C. Plus indicates presence of amino acid.

Amino Acid	ST/ST	CH/CH	AR/AR
Arginine	+	+	+
Cysteine	+	+	+
Glutamine	+		
Glycine	+	+	+
Histidine		+	+
Leucine	+	+	+
Serine	+	+	+
Threonine		+	+
Valine	+	+	

Table 4.

Inversion Homozygotes

Seventy-two hour larva raised at 25° C. Plus indicates presence of amino acid.

Amino Acid	ST/ST	CH/CH	AR/AR
Arginine		+	+
Cysteine	+		+
Glutamine	+		
Glycine	+	+	+
Glutamic Acid		+	
Histidine			+
Leucine	+	+	+
Serine		+	
Threonine		+	
Valine	+		

Table 5.

Inversion Homozygotes

Twenty-four hour pupae raised at 16° C. Plus indicates presence of amino acid.

Amino Acid	ST/ST	CH/CH	AR/AR
Arginine	+	+	+
Aspartic Acid	+		
Cysteine	+	+	
Glutamine	+	+	+
Glycine	+	+	+
Histidine	+	+	
Isoleucine	+	+	+
Lysine	+	+	+
Phenylalanine		+	+
Proline		+	
Threonine		+	+
Tryptophan	+	+	+

Table 6.

Inversion Homozygotes

Twenty-four hour pupae raised at 25° C. Plus indicates presence of amino acid.

Amino Acid	ST/ST	CH/CH	AR/AR
Arginine	+	+	+
Aspartic Acid	+		
Cysteine	+		+
Glutamine	+		+
Glycine		+	
Histidine		+	
Isoleucine		+	
Lysine	+	+	+
Phenylalanine		+	
Proline			+
Threonine		+	
Tryptophan	+		
Valine	+		+

Table 7.

Inversion Homozygotes

Seventy-two hour pupae raised at 16° C. Plus indicates presence of amino acid.

Amino Acid	ST/ST	CH/CH	AR/AR
Arginine	+	+	+
Glycine	+	+	+
Histidine	+	+	+
Leucine	+	+	+
Methionine	+		+
Serine	+	+	+
Theronine			+
Valine		+	

Table 8.

Inversion Homozygotes

Seventy-two hour pupae raised at 25° C. Plus indicates presence of amino acid.

Amino Acid	ST/ST	CH/CH	AR/AR
Arginine	+	+	+
Glycine	+	+	+
Histidine	+	+	
Leucine	+		+
Methionine	+		+
Serine		+	+
Threonine			+
Valine		+	

Table 9.

Inversion Homozygotes

Twenty-four hour imagoes raised at 16° C. Plus indicates presence of amino acid.

Amino Acid	ST/ST	CH/CH	AR/AR
Arginine	+	+	+
Aspartic Acid	+	+	
Glutamine	+	+	+
Histidine		+	+
Leucine	+	+	+
Lysine	+	+	
Threonine	+	+	+

Table 10.

Inversion Homozygotes

Twenty-four hour imagoes raised at 25° C. Plus indicates presence of amino acid.

Amino Acid	ST/ST	CH/CH	AR/AR
Arginine	+	+	+
Asparagine		+	
Aspartic Acid	+	+	+
Glutamine			+
Glutamic Acid	+	+	+
Histidine	+		
Leucine	+		+
Lysine	+	+	+
Threonine	+	+	+
Valine		+	

Table 11.

Inversion Heterozygotes

Zygotes raised at 16° C. Plus indicates presence of amino acid.

Amino Acid	ST/CH	ST/AR	CH/AR
Arginine	+	+	
Cysteine		+	+
Glutamic Acid	+	+	
Histidine		+	+
Isoleucine	+	+	
Serine		+	
Threonine	+	+	+

Table 12.

Inversion Heterozygotes

Zygotes raised at 25° C. Plus indicates presence of amino acid.

Amino Acid	ST/CH	ST/AR	CH/AR
Arginine	+		+
Cysteine	+	+	
Glutamic Acid		+	+
Histidine		+	+
Isoleucine	+		+
Serine		+	
Threonine	+	+	

Table 13.

Inversion Heterozygotes

Seventy-two hour larvae raised at 16° C. Plus indicates presence of amino acid.

Amino Acid	ST/CH	ST/AR	CH/AR
Arginine	+		+
Cysteine	+	+	+
Glycine		+	+
Histidine	+	+	+
Leucine	+	+	+
Lysine	+	+	+
Threonine	+	+	+
Valine	+	+	

Table 14.

Inversion Heterozygotes

Seventy-two hour larvae raised at 25° C. Plus indicates presence of amino acid.

Amino Acid	ST/CH	ST/AR	CH/AR
Arginine	+	+	+
Cysteine	+		+
Glycine	+		+
Glutamic Acid		+	
Histidine	+		
Leucine	+		
Lysine		+	+
Threonine		+	
Valine	+		

Table 15.

Inversion Heterozygotes

Twenty-four hour pupae raised at 16° C. Plus indicates presence of amino acid.

Amino Acid	ST/CH	ST/AR	CH/AR
Arginine	+	+	+
Aspartic Acid	+	+	
Cysteine		+	+
Glutamine	+	+	
Glutamic Acid		+	
Glycine		+	+
Histidine	+		
Leucine		+	+
Threonine	+	+	+
Valine	+	+	+

Table 16.

Inversion Heterozygotes

Twenty-four hour pupae raised at 25° C. Plus indicates presence of amino acid.

Amino Acid	ST/CH	ST/AR	CH/AR
Arginine	+		+
Aspartic Acid	+		+
Cysteine	+	+	
Glutamine		+	+
Glutamic Acid	+		
Glycine	+	+	
Histidine			+
Leucine	+		+
Threonine	+	+	
Valine		+	+

Table 17.

Inversion Heterozygotes

Seventy-two hour pupae raised at 16° C. Plus indicates presence of amino acid.

Amino Acid	ST/CH	ST/AR	CH/AR
Arginine	+		+
Aspartic Acid	+	+	
Cysteine		+	+
Glutamine	+	+	
Glutamic Acid	+	+	
Histidine		+	+
Leucine		+	+
Threonine	+	+	
Valine	+	+	+

Table 18.

Inversion Heterozygotes

Seventy-two hour pupae raised at 25° C. Plus indicates presence of amino acid.

Amino Acid	ST/CH	ST/AR	CH/AR
Arginine	+	+	
Aspartic Acid		+	+
Cysteine	+	+	
Glutamine	+		
Histidine	+		
Leucine		+	+
Threonine		+	+
Valine	+		

Table 19.

Inversion Heterozygotes

Twenty-four hour imagoes raised at 16° C. Plus indicates presence of amino acid.

Amino Acid	ST/CH	ST/AR	CH/AR
Arginine	+	+	+
Aspartic Acid	+	+	+
Cysteine		+	+
Glutamine	+		+
Glutamic Acid			+
Histidine	+		
Isoleucine	+	+	
Leucine	+	+	+
Lysine	+	+	+
Threonine	+	+	+
Valine	+	+	+

Table 20

Inversion Heterozygotes

Twenty-four hour imagoes raised at 25° C. Plus indicates presence of amino acid.

Amino Acid	ST/CH	ST/AR	CH/AR
Arginine	+	+	
Aspartic Acid	+	+	
Cysteine			+
Glutamine			+
Glutamic Acid			+
Glycine	+		
Histidine	+		
Isoleucine	+		
Leucine	+	+	
Lysine	+	+	+
Threonine	+		+
Valine		+	+

APPENDIX B

LIST OF FIGURES

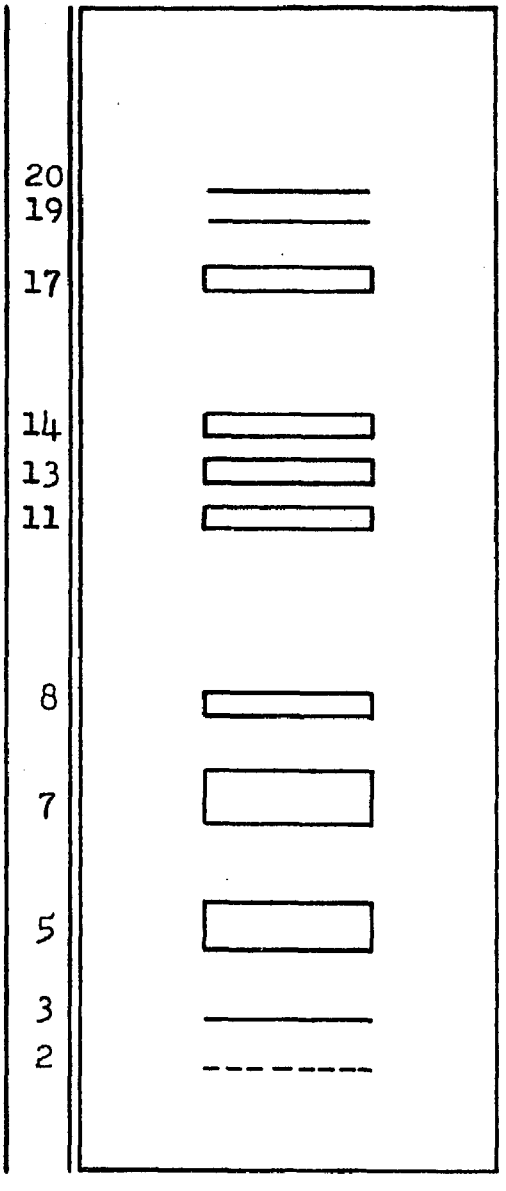
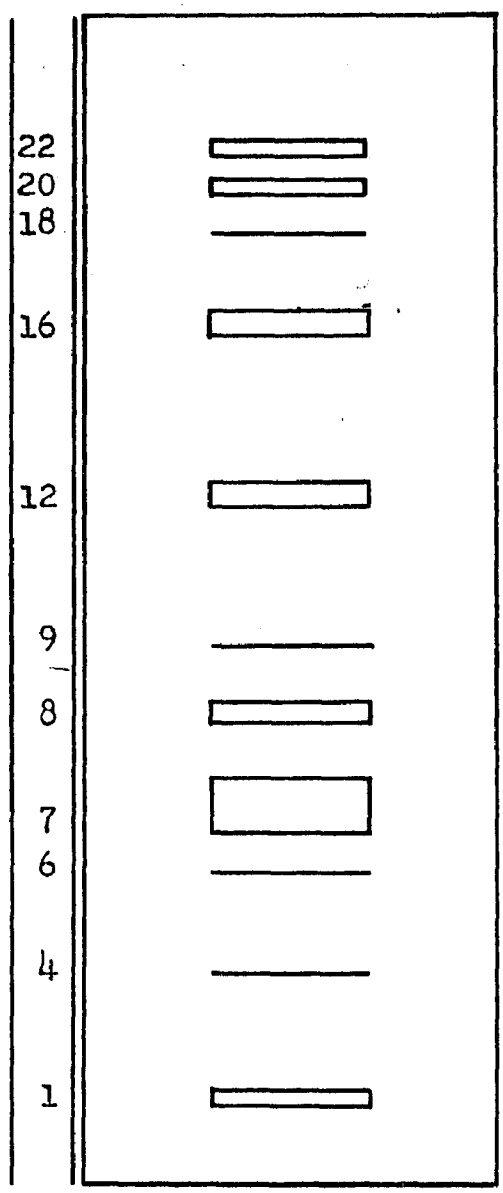


Fig. 1. Protein fraction from ST/ST inversion homozygote raised at 16° C.



Band No.

Fig. 2. Protein fraction from CH/CH inversion homozygote raised at 16° C.

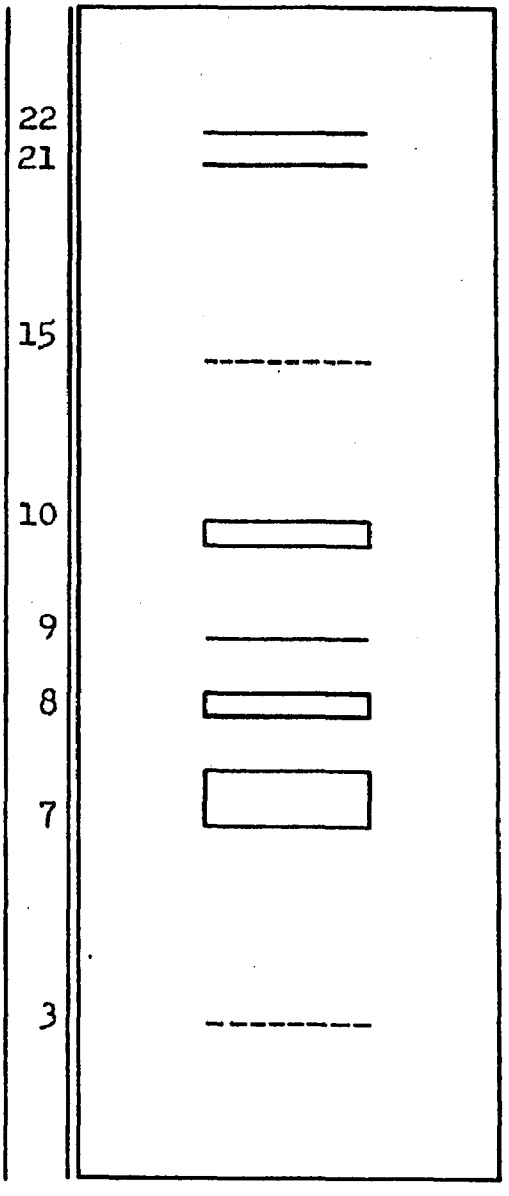
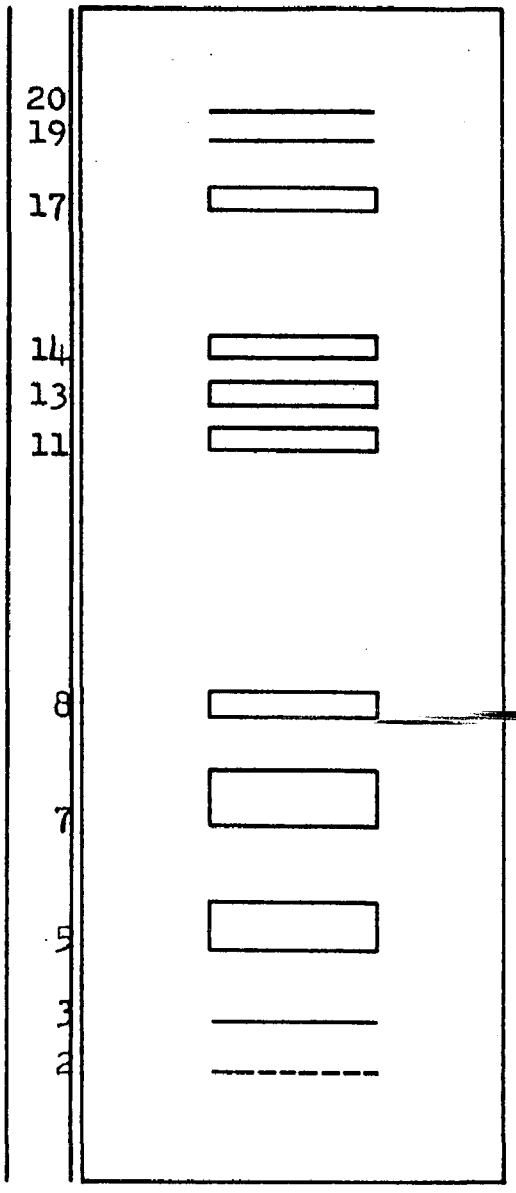


Fig. 3. Protein fraction from AR/AR inversion homozygote raised at 16° C.



Band No.

Fig. 4. Protein fraction from ST/ST inversion homozygote raised at 25° C.

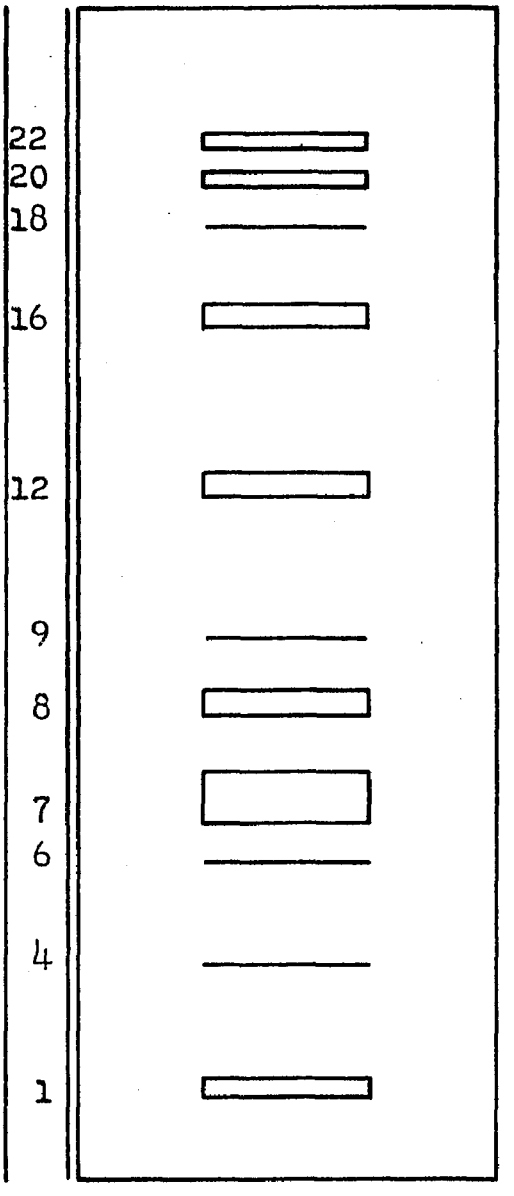


Fig. 5. Protein fraction from CH/CH inversion homozygote raised at 25° C.

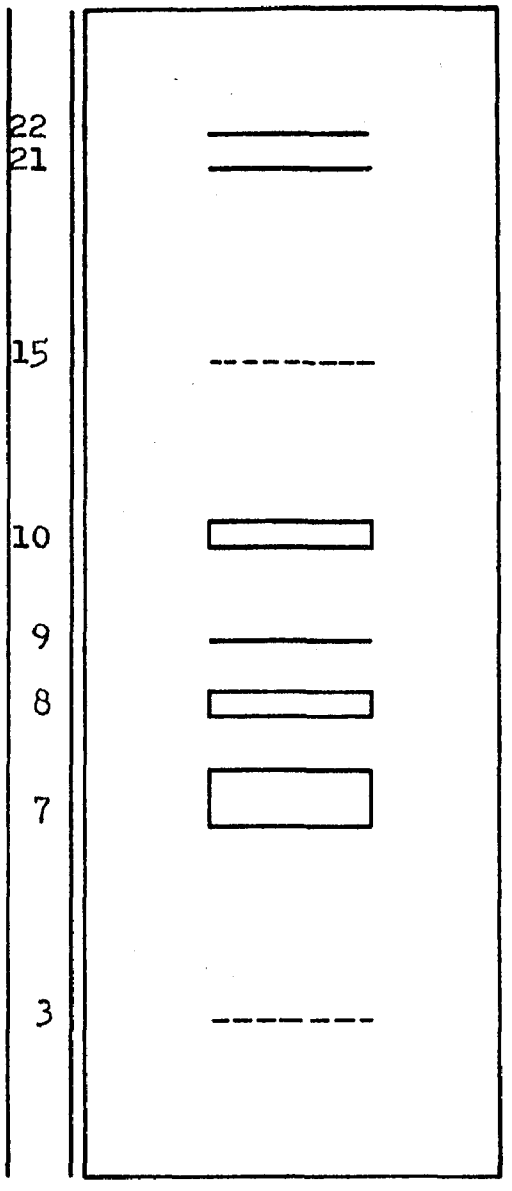


Fig. 6. Protein fraction from AR/AR inversion homozygote raised at 25° C.

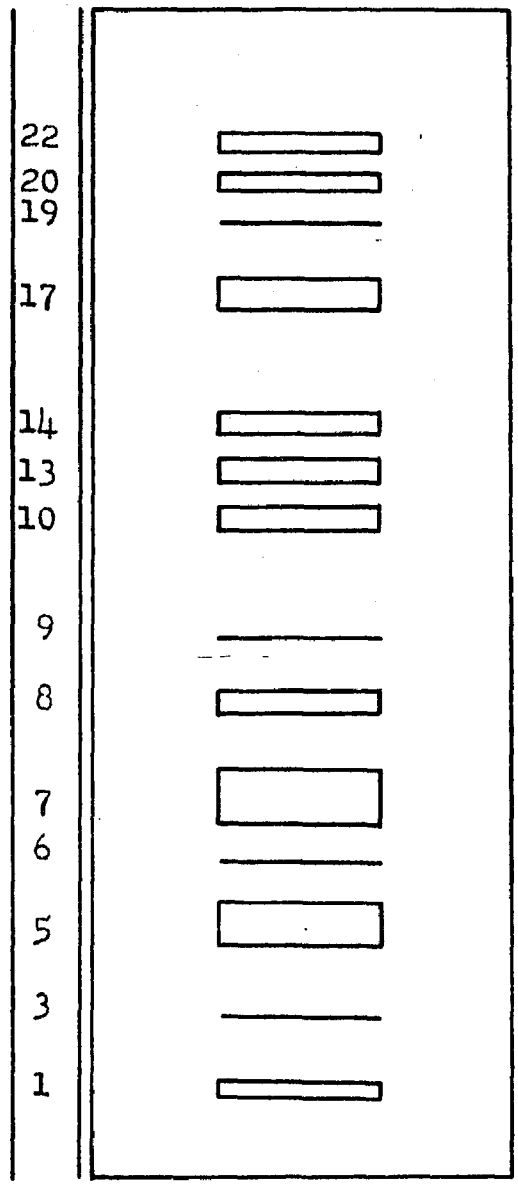
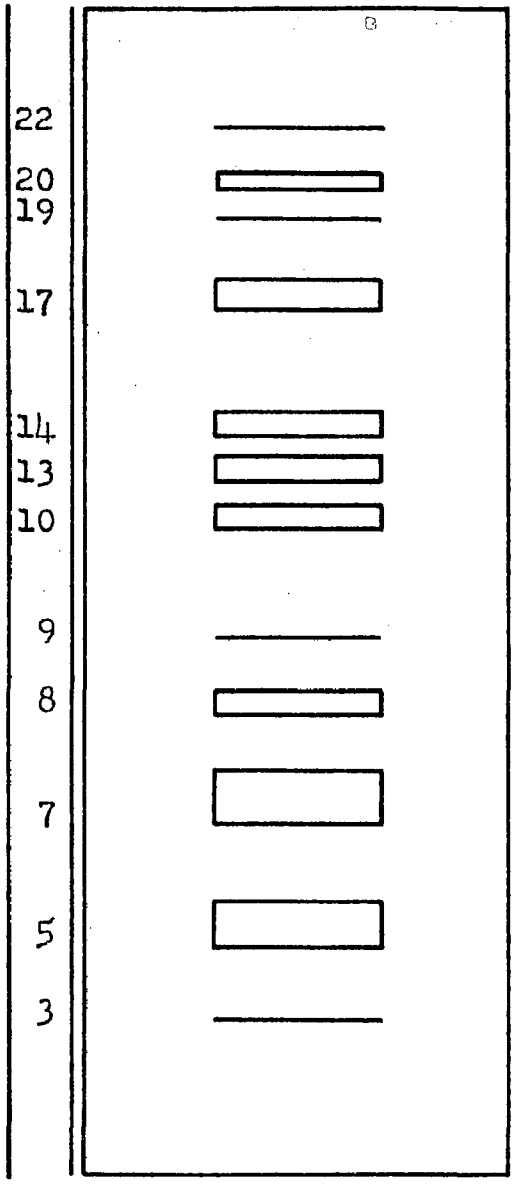


Fig. 7. Protein fraction from ST/CH inversion heterozygote raised at 16° C.



Band No.

Fig. 8. Protein fraction from ST/AR inversion heterozygote raised at 16°C.

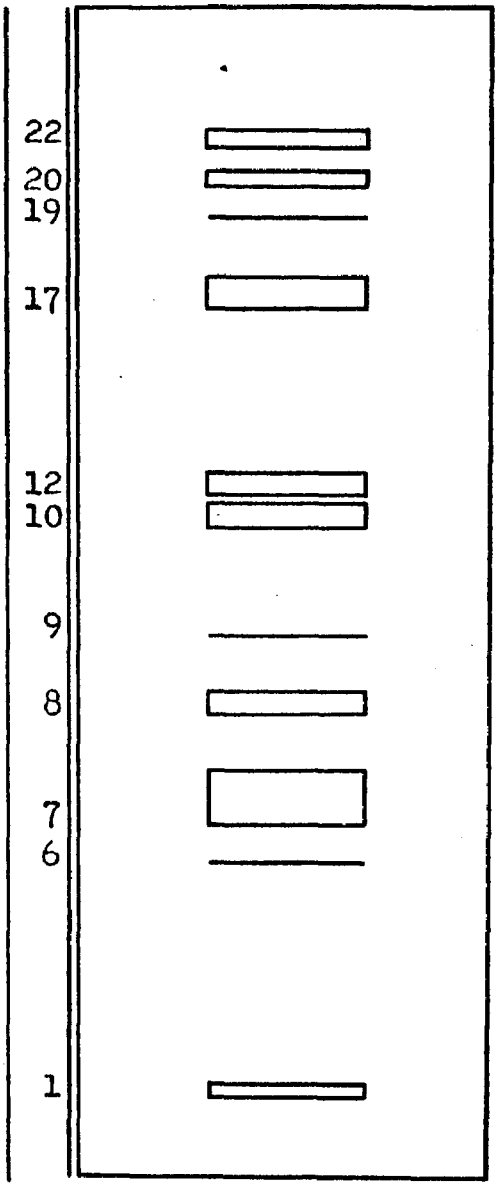


Fig. 9. Protein fraction from CH/AR inversion heterozygote raised at 16° C.

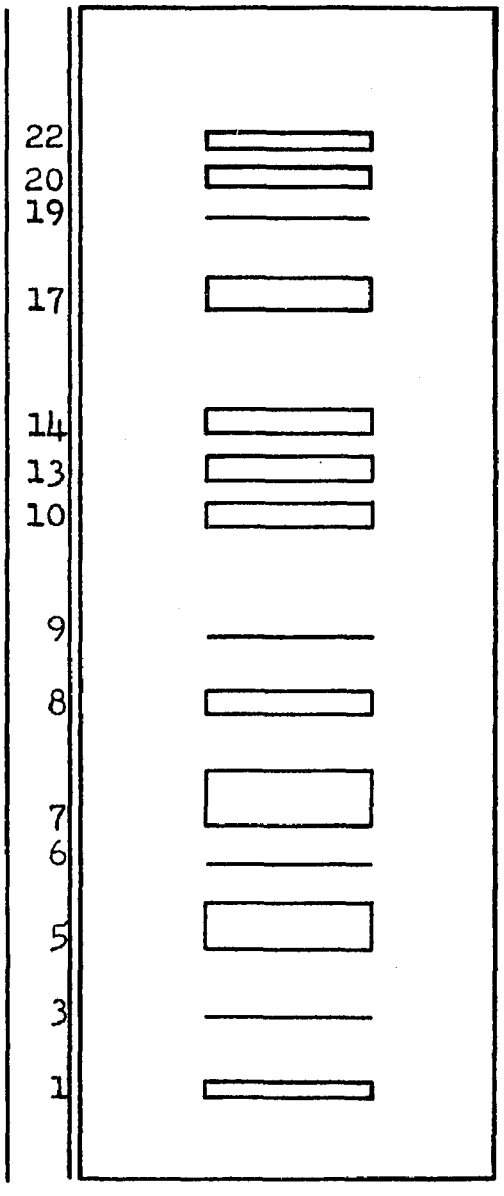


Fig. 10. Protein fraction from ST/CH inversion heterozygote raised at 25° C.

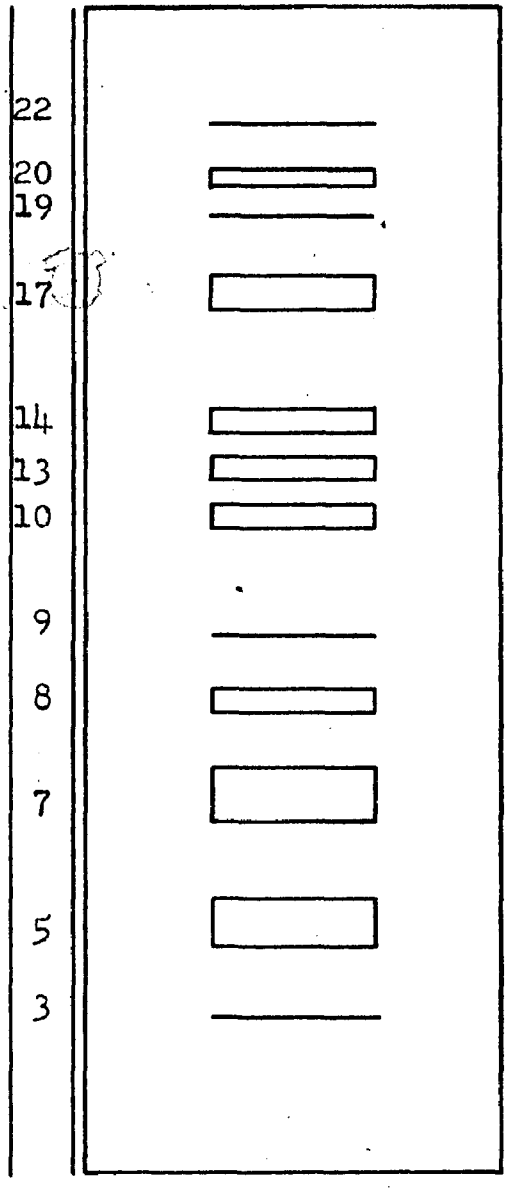


Fig. 11. Protein fraction from ST/AR inversion heterozygote raised at 25° C.

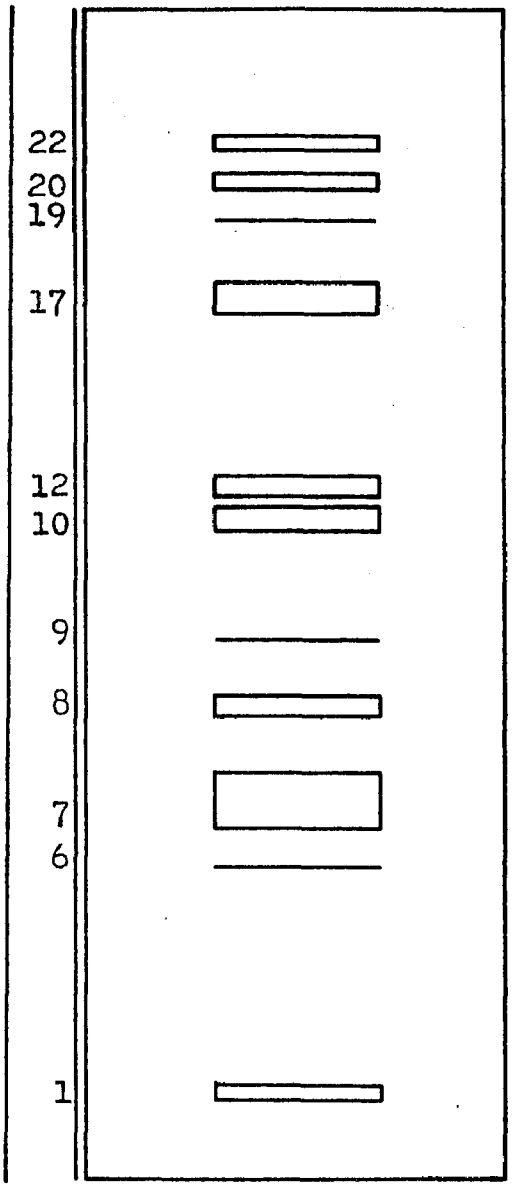


Fig. 12. Protein fraction from CH/AR inversion heterozygote raised at 25° C.

LITERATURE CITED

Allen, S. L., 1960 Inherited variations in the esterases of Tetrahymena. Genetics 45: 1051-1070.

Baglioni, C. 1960 Genetic control of tryptophane pyrrolase in Drosophila melanogaster and Drosophila virilis. Heredity 15: 87-96.

Beadle, G. W. and E. L. Tatum 1941 Genetic control of biochemical reactions in Neurospora. Proc. Natl. Acad. Sci. U. S. 27: 499-506.

_____ 1946 Genes and the chemistry of the organism. Am. Scientist 34: 31-53.

Beckman, L. and F. M. Johnson 1946a Variations in larval alkaline phosphatase controlled by Aph alleles in Drosophila melanogaster. Genetics 49: 829-835.

_____ 1946b Genetic control of amino peptidases in Drosophila melanogaster. Hereditas 51: 221-230.

_____ 1946c Esterase variations in Drosophila melanogaster. Hereditas 51: 212-220.

_____ 1963 Variations of leucine aminopeptidase in pupae of D. melanogaster. Dros. Info. Serv. 38: 69.

_____ 1963 Genetic variations of phosphatase in larvae of D. melanogaster. Dros. Info. Serv. 38: 70.

Benz, G. 1955 Quantitative veranderungen der aminosauren und polypeptide wahrend der entwicklung von Drosophila melanogaster. Arch. Julius Klaus-Stift 30: 498-505.

Carson, H. L. and H. D. Stalker 1949 Seasonal variations in gene arrangement frequencies over a three year period in Drosophila robusta. Evolution 3: 322-329.

Castiglioni, M. C. 1953 Paper chromatography for fluorescent substances in Drosophila melanogaster. Dros. Info. Serv. 27: 87.

Daneel, R. and B. Zimmerman 1954 Uber das Vorkommen und schicksal des kynurenins bei verschidenen Drosophilarassen. Zeitschr. Naturforch. 9b: 788-792.

Doane, W. W. 1966 Disc electrophoresis of ∝-amaylase in individual Drosophila. Dros. Info. Serv. 41:93.

Dobzhansky, Th. 1943 Genetics of natural populations. XII. Temporal changes in the composition of populations of Drosophila pseudoobscura. Genetics 28: 162-186.

_____ 1947a Adaptive changes induced by natural selection in wild populations of Drosophila. Evolution 1: 1-16.

_____ 1947b Genetics of natural populations. XIV. A response of certain gene arrangements in the third chromosome of Drosophila pseudoobscura to natural selection. Genetics 32: 142-160.

_____ 1948 Genetics of natural populations. XVI. Altitudinal and seasonal changes produced by natural selection in certain populations of Drosophila persimilis. Genetics 33: 158-176.

_____ 1950c Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of Drosophila pseudoobscura. Genetics 35: 288-302.

_____ and A. H. Sturtevant 1938 Inversions in the chromosomes of Drosophila pseudoobscura. Genetics 22: 28-64.

_____ and C. Epling 1944 Contributions to the genetics, taxonomy and ecology of Drosophila pseudoobscura and its relatives. Carnegie Inst. Wash. Publ. 554: 1-183.

_____ and Bruce Wallace 1948 Genetics of natural populations. XVI. Altitudinal and seasonal changes produced by natural selection of Drosophila persimilis. Genetics 33: 158-176.

Dubinin, N. P. and G. G. Tiniakov 1945 Seasonal cycles and the concentration of inversions in populations of Drosophila funebris. Am. Nat. 79: 570-572.

_____ 1946b Natural selection and chromosomal variability in populations of Drosophila funebris. Jour. Hered. 37: 39-44.

_____ 1946c Inversion gradients and natural selection in ecological races of Drosophila funebris. Genetics 31: 537-545.

Forrest, H. S., E. Glassman and H. K. Mitchell 1956 Conversion of 2-amino-4-hydroxypteridine to isoxanthopterin in D. melanogaster. Science 124: 725-726.

Glassman, E. and H. K. Mitchell 1959 Mutants of Drosophila melanogaster deficient in Xanthine dehydrogenase. Genetics 44: 153-162.

Hadorn, E. and H. K. Mitchell 1950 Properties of mutants of D. melanogaster and changes during development as revealed by paper chromatography. Proc. Natl. Acad. Sci. U. S. 36: 650-663.

Henning, U. and C. Yanofsky 1963 An electrophoretic study of mutationally altered A protein of the tryptophane synthetase of Escherichia coli. J. Mol. Biol. 6: 16-21.

Heuts, M. J. 1948 Adaptive properties of carriers of certain gene arrangements in Drosophila pseudoobscura. Heredity 2: 63-75.

Horowitz, N. H. 1948 The one gene-one enzyme hypothesis. Genetics 33: 612-613.

_____ 1950 Biochemical genetics of Neurospora. Advances in Genetics 3: 33-71.

_____ and San-Chium Shen 1952 Neurospora tyrosinase. J. Biol. Chem. 197: 512-520.

Hubby, J. L. 1963 Protein differences in Drosophila. I. Drosophila melanogaster. Genetics 48: 871-879.

_____ and L. H. Throckmorton 1965 Protein differences in Drosophila. II. Comparative species genetics and evolutionary problems. Genetics 52: 203-215.

_____ and R. C. Lewontin 1966 A molecular approach to the study of genic heterozygosity in natural populations I. The number of alleles at different loci in D. pseudoobscura. Genetics 54: 577-594.

Hunt, J. A. and V. M. Ingram 1960 Abnormal human haemoglobin IV. The chemical difference between normal human haemoglobin and haemoglobin C. Biochim. Biophys. Acta 42: 409-421.

Ingram, V. M. 1956 A specific chemical difference between the globins of normal human and sickle-cell anaemia haemoglobin. Nature 178: 792-794.

Jacob, F. and J. Monod 1961 Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. 3: 318-356.

Kikkawa, H. 1960 Further studies on the genetic control of amylase in Drosophila melanogaster. Japan. J. Genetics 35: 382-387.

_____ 1963 An agar gel electrophoretic study on amylase in D. virilis. Dros. Info. Serv. 38: 88.

_____ 1965 An electrophoretic analysis of amylase gene in D. simulans. Dros. Info. Serv. 40: 73.

Levitan, M. 1951 Experiments on chromosomal variability in Drosophila robusta. Genetics 36: 285-305.

Lewis, H. W. and H. S. Lewis 1961a Genetic control of dopa oxidase activity in Drosophila melanogaster II. Regulating mechanisms and inter- and intra-strain heterogeneity. Proc. Natl. Acad. Sci. U. S. 47: 78-86.

_____ 1961b Factors on chromosomes II and III involved in the control and regulation of dopa oxidase activity in Drosophila melanogaster. Genetics 46: 878-879.

_____ 1962 Identification of a dominant second chromosome factor regulating dopa oxidase activity in Drosophila melanogaster. Genetics 47: 967.

Lewontin, R. C. and J. L. Hubby 1966 A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of Drosophila pseudoobscura. Genetics 54: 595-609.

McReynolds, M. S. 1967 Homologous esterases in three species of the virilis group of Drosophila. Genetics 56: 527-540.

Nakamara, K., T. Imaizami and Y. Kitazume 1951a Amino acids in D. melanogaster. Dros. Info. Serv. 25: 120.

_____ and M. Takanami 1952 Changes in amino acids during the early development of D. virilis. Dros. Info. Serv. 26: 114.

_____, Y. Kitazume, T. Shiomi and M. Takanami 1953 Biochemical studies of embryonic lethal factors in two strains of D. melanogaster. Dros. Info. Serv. 27: 108.

Painter, T. S. 1934 A new method for the study of chromosome aberrations and the plotting of chromosome maps in Drosophila melanogaster. Genetics 19: 175-178.

Popp, R. A. 1961 Inheritance of different serum esterase patterns among inbred strains of mice. (Abstr.) Genetics 46: 890.

Schwartz, D. 1960 Genetic studies on mutant enzymes in maize. Synthesis of hybrid enzymes by heterozygotes. Proc. Natl. Acad. Sci. U. S. 46: 1210-1215.

Smith, I. 1960 Chromatographic and electrophoretic techniques. 2nd ed. Interscience Publishers, New York, New York.

Smithies, O. 1955 Zone electrophoresis in starch gels. Biochem. J. 61: 629-641.

Spiess, E. 1950 Experimental populations of Drosophila persimilis from an altitudinal transect of the Sierra Nevada. Evolution 4: 14-33.

Strickberger, M. W. 1962 Experiments in genetics with Drosophila. John Wiley and Sons, Inc., New York, New York.

Sturtevant, A. H. 1926 A crossover reducer in Drosophila melanogaster due to inversion of a section of the third chromosome. Biologisches Zentralblatt 46: 697-702.

_____ 1931 Known and probable inverted sections of the autosomes of Drosophila melanogaster. Carnegie Inst. Wash. Publ. 42: 1-27.

Tondo, C. V. and A. R. Cordeiro 1956 Biophysical Genetics I. Paper electrophoresis separation of the eye pigments and other components of Drosophila. Rev. Brasil. Biol. 16: 519-526.

Truter, E. V. 1963 Thin Film Chromatography. Interscience Publishers. New York, New York.

Van der Crone-Gloor 1959 Quantitative investigation of free amino acids and polypeptides during the embryological development of D. melanogaster. Jour. Insect Physiol. 3: 50-56.

Wagner, R. P. and H. K. Mitchell 1955 Genetics and Metabolism. John Wiley and Sons, Inc. New York, New York.

Warters, M. 1944 Chromosomal aberrations in wild populations of Drosophila. Univ. of Texas Publ. 4445: 129-174.

Wright, T. R. F. 1961 The genetic control of an esterase in Drosophila melanogaster. Amer. Zool. 1: 476.

_____ 1963 The genetics of an esterase of Drosophila melanogaster. Genetics 48: 787-801.

Wright, S. and Th. Dobzhansky 1946 Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of Drosophila pseudoobscura. Genetics 31: 125-156.

Yanofsky, C., D. R. Helinski and B. D. Maling 1961 The effects of mutation on the composition and properties of the A protein of Escherichia coli tryptophane synthetase. Cold Spring Harbor Symp. Quant. Biol. 26: 11-24.

_____, B. C. Carlton, J. R. Guest, D. R. Helinski and U. Henning 1964 On the colinearity of gene structure and protein structure. Proc. Natl. Acad. Sci. U. S. 51: 266-272.