

ELECTROPHORETIC VARIATION IN ESTERASES OF *COLIAS EURYTHEME* (PIERIDAE)

F. M. JOHNSON AND JOHN M. BURNS

Genetics Foundation, Dept. Zoology, University of Texas, Austin, Texas
and Dept. Biology, Wesleyan University, Middletown, Connecticut

In recent years, starch gel electrophoresis (Smithies, 1955) has been used to separate multiple molecular forms of enzymes (isozymes—Markert & Møller, 1959) in many plant and animal species. In insects, isozymes of fruit flies of the genus *Drosophila* have received particular attention. The genetics of electrophoretic variation in esterases has been investigated in laboratory strains of *D. melanogaster* Meigen (Wright, 1963; Beckman & Johnson, 1964a; Johnson, 1964). Frequency differences in the alleles controlling esterase variants in *D. ananassae* Doleschall have been found in Samoan island populations (Johnson *et al.*, 1966a). The relative constancy of esterase patterns within species and pattern differences between species has been applied in broad taxonomic comparisons among *Drosophila* species (Johnson *et al.*, 1966b).

In Lepidoptera, the inheritance of esterase variation has been studied in the domesticated silkworm, *Bombyx mori* Linnaeus (Eguchi *et al.*, 1965). The present report is a preliminary account of esterase polymorphism in natural populations of one species of sulfur butterfly, *Colias eurytheme* Boisduval.

MATERIALS AND METHODS

The 140 adults of *C. eurytheme* used in this study were collected in Austin and San Antonio, Texas, from early May to early June, 1966 (Table I). Most (127) were caught in eastern Austin at two localities, one mile apart: Area 1 at the intersection of Chartwell and Lovell drives, and Area 2 at the intersection of Berkman and Broadmoor drives. Both areas were predominantly open, greatly disturbed, and grown over with weeds and grasses. The remaining specimens (13) were caught in disturbed, weedy, open oak-mesquite woodland near Salado Creek to the east of U. S. highway 281 in northern San Antonio. Although *C. eurytheme* was fairly common in the beginning of the sampling period, it became rare toward the end and apparently disappeared by mid-June.

As a rule, live specimens were used and immobilized by pinching the thorax immediately before homogenization; but 13 living specimens were frozen and were satisfactorily electrophoresed at later dates (Table I). To test for esterase activity, butterfly tissues were homogenized by a procedure devised for single specimens of *Drosophila* (Johnson, 1966).

TABLE I
TEXAS SPECIMENS OF *COLIAS EURYTHEME* ANALYZED BY ELECTROPHORESIS

Locality	Date Collected	No. of Specimens Electrophoresed	
		Males	Females
Austin: Area 1	May 7	6	
	May 10	35 ¹	
	May 14	42 ²	8
	May 25	26	1
	May 30	1	
Austin: Area 2	May 28		1
	May 29		1
	May 31	4	1
	June 5		1
San Antonio	May 22	10	3
		124	16

¹ Eleven of these males frozen; electrophoresed on May 23.

² Two of these males frozen; electrophoresed on June 3.

But, because of the comparatively large size of the butterflies, the antennae, legs, or a small section of body were routinely used, instead of whole individuals. After homogenization, the slurry was absorbed in filter paper rectangles which were then inserted into a vertical slot cut in the starch gel. Starch gel electrophoresis was performed horizontally, with a discontinuous system of buffers (Poulik, 1957), for 2.5 hours at a gradient of 20 volts per cm. After electrophoresis, the gels were cut horizontally and the bottom slice stained for esterase activity with α -naphthyl acetate (substrate) and Fast Blue RR salt (dye-coupler) (cf. Beckman & Johnson, 1964a).

RESULTS AND DISCUSSION

Although comparison of antennae, eyes, remainder of head, legs, and thick serial sections of thorax and abdomen revealed some esterase pattern differences, four well-separated esterase zones occurred generally. Observations reported in this paper are based on examination of esterase patterns obtained from mid-abdominal sections of all specimens studied. Individual variation in two of the above-mentioned esterase zones (here called EST C and EST E) was conspicuous in both the Austin and San Antonio samples. Less variation appeared in the other zones.

Figure 1 shows the nature of the electrophoretic variation in zone C. At any one or two of three locations in the gel, either a single or double

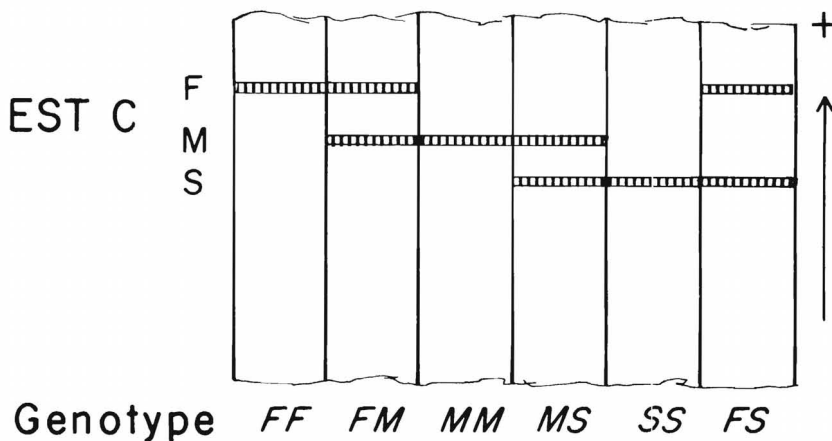


Fig. 1. Part of a gel showing electrophoretic variants in the Esterase C zone. Three presumed alleles, *F*, *M*, and *S*, lead to the production of a single enzyme band when homozygous and a double band when heterozygous. The arrow indicates direction of migration.

band, respectively, was observed in most individuals. This is interpreted as the expression of a tri-allelic system in which the enzyme of fastest mobility is controlled by the allele *Est C^F* of the structural gene *Est C*, and the enzymes of intermediate and slowest mobilities are controlled by alleles *Est C^M* and *Est C^S*. A presumed fourth allele occurred in a few individuals.

A diagrammatic representation of electrophoretic variation in zone E is presented in Figure 2. The presumed homozygotes show a single band, as in EST C, but the presumed heterozygotes show three bands—a band of intermediate mobility in addition to the parental bands. A similar heterozygous pattern has been observed in alkaline phosphatase from *Drosophila* larvae (Beckman & Johnson, 1964b). This type of pattern suggests at least a dimeric active molecule, with the parental bands resulting from combination of like subunits and the hybrid band from unlike subunits. With synthesis of equal numbers, and with random combination of the subunits, a 1 : 2 : 1 (parental : hybrid : parental) distribution of enzymes is expected in heterozygotes. If dimers are equally active, the band densities should follow this distribution; but such a distribution was found to be uncommon upon examination of the presumed heterozygotes. Rather, the presumed hybrid band alone, or, in other cases, both one presumed parental band and the hybrid band were sometimes stained lightly relative to the remainder of the pattern, suggesting unequal rates of synthesis or preferential dimerizing of the subunits.

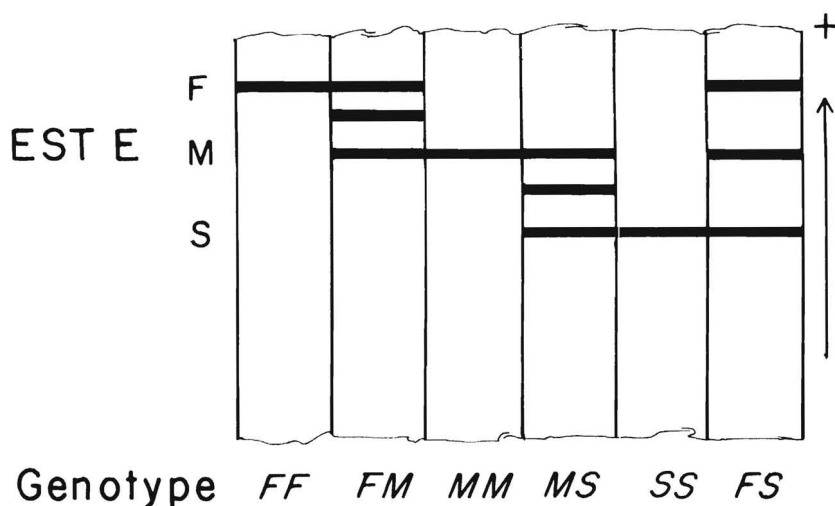


Fig. 2. Part of a gel showing some of the variants in the Esterase E zone. Enzyme bands produced by three alleles (*F*, *M*, and *S*) are pictured in presumed homozygous and heterozygous condition. Heterozygotes have a band of intermediate mobility in addition to the parental bands. The arrow indicates direction of migration.

A striking feature of the variation in EST E is the very large number of apparent alleles. Thirteen distinct parental band positions have been identified in the Austin sample, indicating at least as many alleles. (Only three of the positions appear in Fig. 2.) The small San Antonio sample is also highly variable and includes at least one allele not represented in the Austin sample. At Austin, 77 percent of the individuals are apparently heterozygous for the EST E system; at San Antonio, 83 percent. This large amount of genetic variability, together with differences in rates of synthesis or combination of subunits and/or differences in specificity and activity of the dimeric molecules which may exist, perhaps reflects adaptive flexibility at the molecular level.

A hereditary basis for the variation of EST C and EST E is supported by a partially completed genetic analysis which will be reported elsewhere. A modifier locus may be involved in the expression of the EST E phenotype.

ACKNOWLEDGMENTS

We are indebted to Drs. Wilson S. Stone and Robert K. Selander for providing space in their laboratories for this investigation and to Carmen G. Kanapi, Cynthia Greer, and Susan Rockwood for technical assistance. Roy O. Kendall aided in gathering the San Antonio sample. R. K. Selander read the manuscript and made helpful suggestions. This work

was supported, in part, by Public Health Service Research Grant No. GM 11609 to W. S. Stone and M. R. Wheeler and Training Grant Nos. 2T1-GM-337-06 and GM 00337-07 to R. P. Wagner *et al.*, from the National Institutes of Health.

LITERATURE CITED

- BECKMAN, L., & F. M. JOHNSON, 1964a. Esterase variations in *Drosophila melanogaster*. *Hereditas*, 51: 212-220.
- 1964b. Variations in larval alkaline phosphatase controlled by *Aph* alleles in *Drosophila melanogaster*. *Genetics*, 49: 829-835.
- EGUCHI, M., N. YOSHITAKE, & H. KAI, 1965. Types and inheritance of blood esterase in the silkworm, *Bombyx mori* L. *Japan. Jour. Genet.*, 40: 15-19.
- JOHNSON, F. M., 1964. A recessive esterase deficiency in *Drosophila*. *Jour. Heredity*, 55: 76-78.
1966. Rapid single fly homogenization for the investigation of *Drosophila* isozymes. *Drosophila Inf. Serv.*, 41: 193-194.
- JOHNSON, F. M., C. G. KANAPI, R. H. RICHARDSON, M. R. WHEELER, & W. S. STONE, 1966a. An analysis of polymorphisms among isozyme loci in dark and light *Drosophila ananassae* strains from American and Western Samoa. *Proc. Nat. Acad. Sci.*, 56: 119-125.
- 1966b. An operational classification of *Drosophila* esterases for species comparison. *Univ. Texas Publ.*, in press.
- MARKERT, C. L., & F. MØLLER, 1959. Multiple forms of enzymes: tissue, ontogenetic, and species specific patterns. *Proc. Nat. Acad. Sci.*, 45: 753-763.
- POULIK, M. D., 1957. Starch gel electrophoresis in a discontinuous system of buffers. *Nature*, 180: 1477-1479.
- SMITHIES, O., 1955. Zone electrophoresis in starch gels: group variations in the serum proteins of normal human adults. *Biochem. Jour.*, 61: 629-641.
- WRIGHT, T. R. F., 1963. The genetics of an esterase in *Drosophila melanogaster*. *Genetics*, 48: 787-801.

INTERNATIONAL COMMISSION ON ZOOLOGICAL NOMENCLATURE

A.(n.s.)75

ANNOUNCEMENT

Required six-months' public notice is given on the possible use of plenary powers by the International Commission on Zoological Nomenclature in connection with the following names, listed by Case Number (see *Bull. zool. Nomencl.* 23, pt. 4, 14 October 1966):

1742. Type-species for *Cosmopterix* Hübner, 1825 (Lepidoptera)
1745. Suppression of *Macrochoeta* Macquart, 1851 (Diptera)
1747. Emendation of STENOPODINAE Stål, 1859, to STENOPODINAE (Hemiptera)
1748. Suppression of *Scoptes* Hübner, [1819] (Lepidoptera)
1758. Type-species for *Neolycaena* de Nicéville, 1890 (Lepidoptera)