

technique, considering also the morphological characteristics of the different populations and their subspecific systematic positions. Our material included samples of 6 specimens (2♂♂, 4♀♀) of *C. c. balcanicus* collected from the vicinity of Sofia at about 550 m altitude and from the vicinity of Koprivstitsa, at 1050 m altitude; 5 specimens (3♂♂, 2♀♀) of *C. c. martinoi* collected from Rhodopa mountains at 2600 m altitude; 6 specimens (2♂♂, 4♀♀) of *C. c. lascarevi* collected from the Danube plain at about 200 m altitude; 6 specimens (1♂, 5♀♀) of *C. c. ssp. nova* collected from Dobroudja at 150 m altitude; and 5 specimens (2♂♂, 3♀♀) of *C. citellus* collected from Trakia at about 300 m altitude whose subspecific position is not established but whose morphological characteristics are similar to those of *C. c. lascarevi*.

All the specimens studied have a diploid number of 40 chromosomes comprising 5 pairs of submetacentric, 7 pairs of subtelocentric, 5 pairs of acrocentric and 2 pairs of metacentric autosomes (figure 1). The chromosomes are classified according to Levan et al.<sup>8</sup>. The autosomal karyotype did not differ detectably from that described by other authors<sup>4-7</sup> and will not be discussed further. The only variation of major significance is in the morphology of the X chromosomes. Variations in the morphology of the sex chromosomes are not usual among rodents. They have been found in *Rattus*<sup>9</sup>, *Peromyscus*<sup>10</sup>, *Akodon*<sup>11</sup>, *Mus*<sup>12</sup>, *Spermophilus*<sup>13</sup>, *Neotoma*<sup>14</sup>, *Tatera*<sup>15</sup>, *Zygodontomys*<sup>16</sup>, *Bandicota*<sup>17,18</sup>, *Oryzomys*<sup>19</sup>. The morphology of the X chromosomes is apparently the same in the 3 subspecies *citellus*, *balcanicus*, *martinoi* and in the individuals of the subspecies *nova* from Dobroudja. The X chromosome is a medium-sized submetacentric (figure 1). But in all specimens of *C. c. lascarevi* and in the individuals of the subspecies from Trakia, the X chromosome is a medium-sized acrocentric (figure 2). The X chromosome variants in these cases differ

only in morphology but not in size. Thus the difference may be due to a pericentric inversion. The Y chromosome is the smallest element of the complement, measuring about 0.5 µm in all subspecies, and it appears to be acrocentric.

- 1 The authors wish to express their sincere thanks to Prof. Ts. Peshev (Sofia University, Department of Zoology) for providing and identifying the subspecies and also to D. Todorova for her skilful technical help.
- 2 Ts. Peshev and Z. Rashev, *Annls Univ. Sofia* 66, 256 (1974).
- 3 Ts. Peshev, *Bull. Inst. Zool., Sofia*, 4/5, 1955.
- 4 S. Zivkovic, *Arch. biol. Sci.* 17, 17 (1966).
- 5 S. Zivkovic, B. Soldatovic, M. Milosevic and I. Savic, *Zool. Anz.* 181, 181 (1968).
- 6 I. Savic, M. Milosevic and S. Zivkovic, *Arch. biol. Sci.* 23, 33 (1971).
- 7 N.N. Vorontsov and E.A. Ljapunova, II. Union Mammalogy Conference, Novosibirsk 1969.
- 8 A. Levan, K. Fredga and A.H. Sanberg, *Hereditas* 52, 211 (1964).
- 9 D.A. Hunderford and P.C. Nowell, *J. Morphol.* 113, 275 (1963).
- 10 T.C. Hsu and F.E. Arrighi, *Cytogenetics* 7, 417 (1963).
- 11 N.O. Bianchi, O.A. Reig, O.J. Molina and F.N. Dulout, *Evolution* 25, 724 (1971).
- 12 R. Matthey, *Cytogenetics* 6, 168 (1970).
- 13 C.F. Nadler and C.E. Hughes, *Science* 151, 579 (1965).
- 14 D.H. Wurster, J.R. Snapper and K. Benirschke, *Cytogenetics* 10, 153 (1971).
- 15 S.R.V. Rao, V.C. Shahand and S. Champaka, *Chromosoma* 23, 304 (1968).
- 16 Y. Yonenaga, O. Frota-Pessoa, S. Kasahara and E.I. Cardoso de Almeida, *Cienc. Cult., Maracaibo* 28, 202 (1976).
- 17 S. Pathak, *Experientia* 28, 221 (1972).
- 18 T. Sharma and R. Raman, *Chromosoma* 41, 75 (1973).
- 19 M. Gallardo and A. Gonzales, *Experientia* 33, 312 (1977).

## Electrophoresis of proteins in three populations of *Ophryotrocha labronica* La Greca e Bacci 1962 (Annelida Polychaeta)

C. Robotti

Istituto di Zoologia, Università di Torino, via Accademia Albertina 17, I-10123 Torino (Italy), 15 September 1978

**Summary.** Different degrees of similarity are found between the electrophoretic patterns of proteins of *Ophryotrocha labronica* populations collected in 3 different localities.

Life histories and sex conditions of samples of *Ophryotrocha labronica* collected in different localities and in different years suggest that this comprehensive species is composed of highly heterogeneous populations<sup>1-3</sup>. The same has been observed in other marine species, belonging to the genera *Crepidula*<sup>4</sup>, *Littorina*<sup>5</sup>, *Jaera*<sup>6</sup>, *Tisbe*<sup>7</sup> and *Capitella*<sup>8</sup>.

Colonna<sup>9</sup> showed very significant differences in the masticatory apparatus between females collected in Venice (VE), Naples (NA IV) and Faro, Portugal (FA).

The electrophoretic mobility of general proteins from the 3 populations is now investigated in an attempt to cast more light on their relationships within the *Ophryotrocha labronica* group. The technique of acrylamide gel electrophoresis was employed in the present research.

Each sample consisted of 20 adult specimens, a number that, from preliminary essays, proved most suitable to obtain a reliable and repeatable picture of the general proteins.

Homogenization was performed in 0.5 ml glass containers employing a dental drill<sup>10</sup>, in 40 µl Tris-glycine 0.5 mM

buffer, pH 8.3. 20 µl of glycerol-bromophenol blue were added to clear supernatant after centrifugation at 14,000 × g for 15 min, and 20 µl of such extract were used for electrophoresis.

This was performed according to the disc method of Davis<sup>11</sup> in glass tubes 60 mm in length and 2 mm in diameter. A glycine-Tris buffer of pH 8.3 was used for the run, and electrophoresis was carried out for 1 h with a voltage of 50 V for 20 min, 100 V for 10 min, 200 V for 30 min. The gels were then removed from the glass tubes and stained overnight in coomassie blue. Destaining was

Similarity matrix for the VE-FA-NA IV populations

	VE	FA	NA IV
VE	X	0.2386	0.2844
FA	0.7614	X	0.2752
NA IV	0.7156	0.7248	X

Lower values are the similarity coefficients of Jaccard ( $S_j$ ), upper values are distances ( $1-S_j$ ).

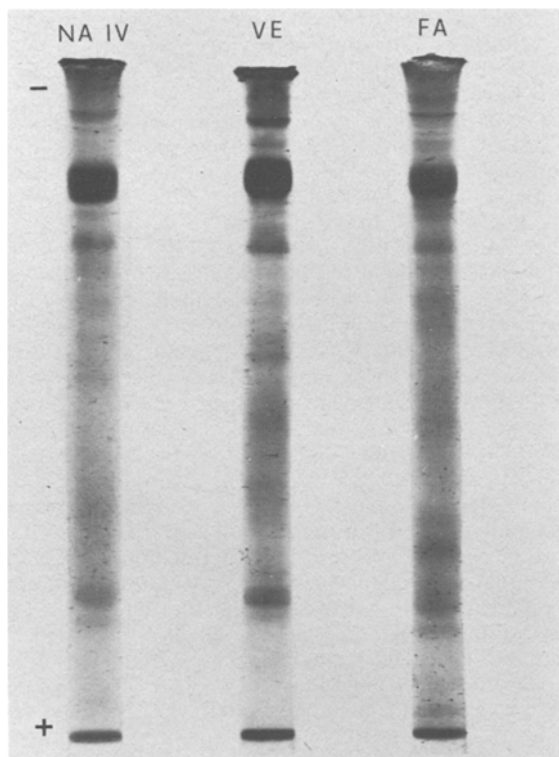


Fig. 1. General Protein Electropherograms of *Ophryotrocha labronica* populations. VE, Venice; FA, Faro; NA IV, Naples.

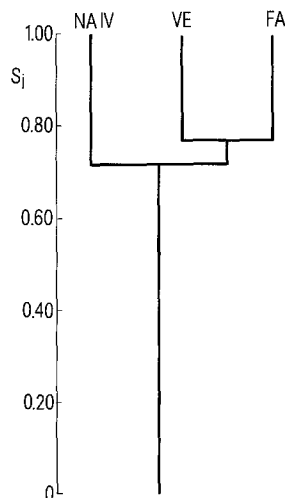


Fig. 2. UPGMA Pherogram derived from similarity matrix of the table.

carried out by leaching with coomassie blue solvent (25% ethanol, 8% acetic acid), and gels were preserved in 7% acetic acid containing a small amount of dye. The relative mobilities of the different protein fractions are shown in figure 1.

Since individual pherograms were not available, it was not possible to use Nei's method<sup>12</sup>, and the similarity coefficient of Jaccard was employed to compare the 3 patterns<sup>13,14</sup>.

The mobility of each protein fraction was plotted on a grid and the number of classes for each band was determined, coding one if the band was present, zero if it was absent<sup>15</sup>. The table lists such similarity coefficients for pairs of populations. The highest value is 0.7614 and the smallest distance is 0.2844 for pairs of VE-FA populations. From this similarity matrix the pherogram of figure 2 was drawn, using the UPGMA method<sup>16</sup>. The cophenetic correlation coefficient between the pherogram and the cophenetic matrix that can be derived from it is 0.999<sup>16</sup>.

As shown by Colonna<sup>9</sup>, the populations from Venice and Faro are the most similar, while the Naples one is rather distant from the former 2. These results also give rough but interesting information about the amount of similarity: while the pair VE-FA has about 76% of genotype in common, NA IV share with the other 2 populations only 71% of genotype. Investigations on another species of the same genus, *O. puerilis* showed that, according to unpublished data, there is about 66% similarity between different species. The technique used, with some modifications necessary to perform individual electrophoresis, will soon allow us to characterize more exactly the genetic structure of *O. labronica* populations, with regard to genic frequencies of some enzymes.

- 1 B. Åkesson, Pubbl. Staz. Zool. Napoli 39, 377 (1975).
- 2 M. La Greca and G. Bacci, Boll. Zool. 29, 18 (1962).
- 3 R. Zunarelli, Boll. Zool. 29, 417 (1962).
- 4 W.R. Coe, J. Morph. 84, 383 (1949).
- 5 S.A. Mileikovsky, Mar. Biol. 30, 129 (1975).
- 6 C. Bocquet, in: Fifth European Marine Biology Symposium, p. 131. Ed. B. Battaglia. Piccin, Padova 1972.
- 7 B. Volkmann-Rocco and B. Battaglia, in: Fifth European Marine Biology Symposium, p. 67. Ed. B. Battaglia. Piccin, Padova 1972.
- 8 J.P. Grassle and J.F. Grassle, Science 192, 567 (1976).
- 9 F. Colonna, Experientia 34, 1565 (1978).
- 10 D. Eichner, Experientia 22, 620 (1966).
- 11 J.B. Davis, Ann. N. Y. Acad. Sci. 121, 404 (1964).
- 12 M. Nei, Am. Nat. 106, 283 (1972).
- 13 P. Jaccard, Bull. Soc. vaud. Sci. nat. 44, 223 (1908).
- 14 P.H.A. Sneath, J. gen. Microbiol. 17, 184 (1957).
- 15 D. Borden, E.T. Miller, G.S. Whitt and D.L. Nanney, Evolution 31, 91 (1977).
- 16 P.H.A. Sneath and R.R. Sokal, Numerical Taxonomy, Freeman, S. Francisco 1973.

### Laboratory observations on pupae on the medfly *Ceratitis capitata* (Wied.)

A. Shoukry

Plant Protection Laboratory, National Research Centre, Dokki, Cairo (Egypt), 17 July 1978

**Summary.** Laboratory observations on pupae of *Ceratitis capitata* showed that most adult emergence took place in the morning between 06.00 and 09.00 h. Pupae succeeded to emerge when covered with sand up to a depth of 30 cm. It was not possible to differentiate between sexes by pupal weight.

In recent years, there have been several studies on the possibility of applying the sterile insect technique against the medfly *Ceratitis capitata* (Wied.) in different parts of the world. In Egypt, the ecological factors affecting the

control of *C. capitata* by the sterile male technique and the biological effects of gamma irradiation on adult fecundity and longevity were investigated<sup>1-4</sup>. The present paper furnishes some additional laboratory observations on the pupae