Genet. Res., Camb. (1968), **11**, *pp.* 179–182
With 3 text-figures
Printed in Great Britain

Polymorphism of plasma esterases in flounder and plaice

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(*Received* 8 *November* 1967)

Genetic control of electrophoretic variants of esterases by co-dominant autosomal alleles has been demonstrated in various organisms (Shaw, 1965; Grunder, Sartore & Stormont, 1965; Gahne, 1966; Oki & Miura, 1966; Tucker, Suzuki & Stormont, 1967; Burns & Johnson, 1967). In a number of cases, including a species of freshwater fish, the char (*Salvelinus alpinus* L.) (Nyman, 1967), production of multiple esterase zones under the control of one allelle was described (Allen, 1961; Popp & Popp, 1962; Tashian & Shaw, 1962; van Asperen, 1964; Grunder *et al.* 1965; Gahne, 1966). This paper deals with polymorphism of plasma esterases in two species of marine fish: flounder (*Pleuronectes flesus* L.) and plaice (*Pleuronectes platessa* L.).

Blood samples were collected in heparin and used fresh or fresh-frozen. Starch-gel electrophoresis was carried out with a buffer system modified after Aronsson & Grønwall (1957) as used in earlier studies of plaice transferrins (de Ligny, 1967). Esterase activity was detected using α-naphthylacetate (5 ml of 1% solution in cetone/250 ml distilled water) as a substrate and Fast Blue RR (250 mg) as a dye coupler. Incubation for 10–15 min at 25 °C was sufficient for the development of the major esterase zones. Apart from a stained zone along the buffer boundary and an occasional weak band immediately behind it, in some animals, esterase activity appeared to be confined to a region about two-thirds of the migration distance of the albumin zone. In both species the enzyme activity in this region was found to be located in groups of three bands, either appearing as single units or arranged in patterns with five or six bands. The bands are well defined and rather evenly spaced from each other. They vary in intensity and occasionally there is an additional anterior weak band. On the assumption that each group of three bands is produced under the control of one allele, and taking into account the regularity of the differences in their migration rate, they were labelled as illustrated in Fig. 1.

Figure 2 displays diagrammatically the types which were observed during study of blood from more than 500 flounders and 600 plaice. In addition, six flounders were found with a rather blurred pattern, migrating at a rate comparable to the G and H groups. This type tentatively was called GH. It can be seen from Fig. 1 that occurrence of presumably heterozygous patterns with five bands can be explained by identical migration of one band of each of the corresponding homozygote types.

Due to the rarity of the only set of bands that appears to occur in both flounder and plaice, the G group, it was not possible to compare its migration in the same gel. At present no relationship between the esterase groups of both species other than their relative migration velocity is postulated.

A significant difference in staining intensity of the individual bands was noticed

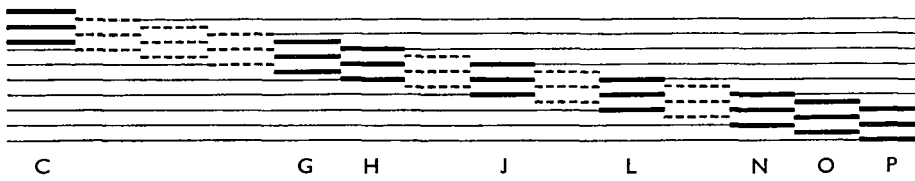

Fig. 1. Nomenclature of groups of three bands, based on their relative migration velocity. Heavy solid lines: types observed; broken lines: positions of hypothetical intermediate types.

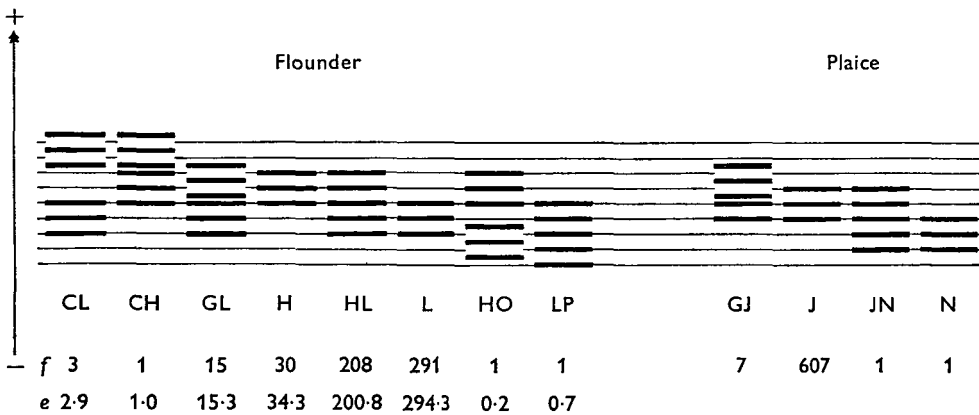

Fig. 2. Diagram of the esterase types observed, the numbers of fish in which they occurred (*f*) in 550 flounder and 616 plaice, and the numbers expected according to the Hardy–Weinberg law (*e*).

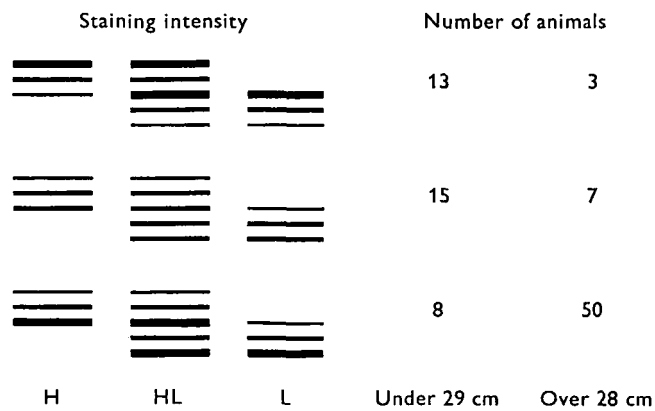

Fig. 3. Variations in staining intensity of H, HL and L types in flounder, and their distribution in two groups of animals of different length.

https://doi.org/10.1017/S0016672300011344 Published online by Cambridge University Press

when flounders of different lengths were compared. It is illustrated in Fig. 3 with regard to the patterns most frequently encountered. The numbers of animals from two groups of different length, showing the various staining patterns, are given alongside.

Analysis of the frequencies of the various types observed in flounder, as presented in Fig. 1, supports the hypothesis of genetic control of the esterases by codominant multiple alleles, each governing the appearance of a group of three bands. When the frequencies of the hypothetical genes were calculated, the numbers of animals showing the various phenotypes were found to be in good agreement with the numbers expected according to the Hardy–Weinberg law. Including the presumed GH phenotype in the calculation and combining classes in which less than five animals were found, the χ^2 value is 0·86 (D.F. 5).

With respect to the production of three bands under the control of one allele the following can be said. The possibility of interaction of macromolecules and buffer components, as described by Cann and referred to by Grunder *et al.* (1965), was excluded following the procedure described by Cann & Goad (1965). Individual bands were isolated from the unstained half of the gel and subjected to a second run. They were found to migrate at their original speed and did not show any further separation. Both dimerization (Shaw, 1965; Grunder *et al.* 1965; Burns & Johnson, 1967) and polymerization (Shaw, 1965; Allen, 1961; Lush, 1966) of basic allelic products have been suggested to account for the appearance of 'hybrid' esterase zones in heterozygotes and multiple zones in homozygote individuals.

A composite structure of the esterase bands, involving one component under control of a mutated locus, could account for the variant patterns observed. The number and relationship of the components await further elucidation, by means of biochemical and genetical studies.

SUMMARY

Esterase activity was detected in starch-gel electropherograms of plasma samples from two species of teleost: flounder and plaice. In both species polymorphism was found. In flounder its genetic origin was indicated by population genetical data. Differences in staining intensity of the individual bands were found to be correlated with length of the animals.

I am indebted to Ir. W. Welling, Laboratory for Research of Insecticides, Wageningen, for helpful criticism. The suggestions made by Dr F. W. Robertson, Institute of Animal Genetics, Edinburgh, who kindly read the manuscript, are gratefully acknowledged.

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