Loukas, M. and C.B. Krimbas. Agricultural College of Athens, Greece. Isozyme techniques in D. subobscura.

The electrophoretic techniques for the detection of 26 polymorphic genes in D. subobscura are given here. All electrophoretic assays were performed on horizontal starch gel electrophoresis (starch from Connaught Med. Lab., Toronto). The following table gives the buffer solutions used for gel preparation, for filling the electrode compartments and for developing the gels. Also given are the substrates, dyes, coenzymes, coupling enzymes and inorganics added to the staining solutions.

Key to the symbols used:

A: 0.25 M TRIS, pH 8.6
A1: 1/5 dilution of buffer A
A2: 4/5 dilution of buffer A
B: 0.687 M TRIS + 0.157 M citric acid, pH 8.0
C: 0.135 M TRIS + 0.045 M citric acid, pH 7.0
C1: 1/3.3 dilution of buffer C
C2: 1/15 dilution of buffer C
D: 0.18 M TRIS + 0.1 M boric acid + 0.004 M EDTA.2Na, pH 8.6
D1: 1/4 dilution of buffer D
E: 0.1 M TRIS + 0.1 M maleic acid + 0.01 M EDTA.2Na + 0.001 M $MgCl_2$, pH 7.4
E1: 1/10 dilution of buffer E
F: 0.05 M TRIS + 0.05 M maleic acid, pH 7.4
F1: 1/5 dilution of buffer F
G: 0.05 M TRIS + 0.007 M citric acid, pH 8.6
H: 0.76 M boric acid + 0.2 M LiOH, pH 8.2
H1: 1/4 dilution of buffer H
I: 0.076 M TRIS + 0.005 M citric acid, pH 8.7
J: 0.3 M boric acid + 0.062 M NaOH, pH 8.4
K: 0.017 M Na_2HPO_4 + 0.049 M KH_2PO_4, pH 6.4
L: 0.023 M citric acid + 0.053 M Na_2HPO_4, pH 5.2
M: 0.023 M citric acid + 0.06 M Na_2HPO_4, pH 6.1
N: 0.005 M citric acid + 0.076 M TRIS + 0.005 M $MgCl_2$, pH 8.7
O: 0.087 M TRIS + 0.0087 M boric acid + 0.001 M EDTA.2Na, pH 9.0
P: 0.1 M TRIS - HCl, pH 8.5
Q: 0.1 M TRIS - HCl, pH 8.0
R: 0.1 M TRIS - HCl, pH 7.5
S: 0.025 M Na_2HPO_4, pH 7.6
T: 0.05 M NaOH, pH 5.2 (with acetic acid)
U: 0.1 M TRIS - HCl, pH 7.1

Abbreviations used:

Est: esterase
Lap: leucine aminopeptidase
Odh: octanol dehydrogenase
Idh: isocitric dehydrogenase
Ao: aldehyde oxidase
Xdh: xanthine dehydrogenase
G-6-PD: glucose-6-phosphate dehydrogenase
6-PGD: 6-phosphogluconate dehydrogenase
ME: malic enzyme
Pgm: phosphoglucomutase
α-GPD: α-glycerophosphate dehydrogenase
Mdh: malate dehydrogenase
Pept: peptidase
Hk: hexokinase
Phi: phosphoexose isomerase
Aph: alkaline phosphatase
Acph: acid phosphatase
Adh: alcohol dehydrogenase
Ald: aldolase
Fum: fumarase
GA-3-PD: glyceraldehyde-3-phosphate dehydrogenase
DCIP: 2,6-dichlorophenolindophenol
NBT: nitro blue tetrazolium
PMS: phenazine methosulfate
NAD: nicotinamide adenine dinucleotide
NADP: nicotinamide adenine dinucleotide phosphate
ATP: adenosine triphosphate

Genetical information concerning the above mentioned enzyme polymorphisms, the localization of the corresponding genes and mapping data are given by Loukas et al. (J. Heredity, 70:17-26).

Enzyme Techniques

Allozyme assays, bridge and gel buffers and constituents of enzyme stains are indicated.

Enzyme	Gel buffer	Bridge buffer	Staining solution	Substrate(s)	Dyes	Coenzymes	Coupling enzymes	Inorganics
Est-3	9G : 1H	H1	K	α-naphthyl acetate β-naphthyl acetate	Fast blue BB	-	-	Mg^{++}, Mn^{++}
Est-5 & Est-7	9G : 1H	H1	K	α-naphthyl acetate	Fast blue RR	-	-	Mg^{++}, Mn^{++}
Est-9	9G : 1H	H1	M	α-naphthyl acetate l-leucyl-β-naphthylamide	Fast blue RR Black K	-	-	Mg^{++}, Mn^{++}
Lap	9G : 1H	H1	L	l-leucyl-β-naphthylamide	Black K	-	-	Mg^{++}, Mn^{++}
Odh	I	J	P	n-octanol	NBT, PMS	NAD	-	-
Diaphorase (*)	I	J	Q	NADH	DCIP	-	-	-
Idh	I	B	Q	Isocitric acid	NBT, PMS	NADP	-	Mn^{++}
Ao	A1	A	P	Benzaldehyde	NBT, PMS	-	-	-
Xdh	A1	A	R	Hypoxanthine	NBT, PMS	NAD	-	-
G-6-PD	A1	A2	R	Glucose-6-phosphate	NBT, PMS	NADP	-	-
6-PGD	A1	A2	P	6-phosphorogluconate	NBT, PMS	NADP	-	-
ME	E1	E	P	Malic acid	NBT, PMS	NADP	-	-
Pgm	E1	E	Q	Glucose-1-phosphate (G-1, 6-DP)	NBT, PMS	NADP	G-6-PD	Mg^{++}
α-GPD	E1	E	P	α-glycerophosphate	NBT, PMS	NAD	-	-
Mdh	E1	E	Q	Malic acid	NBT, PMS	NAD	-	-
Pept-1 & Pept-3 (*)	D1	D	S	L-leucyl-L-tyrosine	3-amino-9 ethyl carbazole	-	Amino acid oxidase (snake venom)	-
Hk-1	D1	D	P	Glucose, ATP	NBT, PMS	NADP	G-6-PD	Mg^{++}
Phi (*)	D1	D	Q	Fructose-6-phosphate	NBT, PMS	NADP	G-6-PD	Mg^{++}
Aph	N	J	N	α-naphthyl phosphate	Fast blue RR	-	-	Mg^{++}, Mn^{++}
Acph	C1	C	T	α-naphthyl phosphate	Fast blue RR Black K	-	-	Mg^{++}, Mn^{++}
Adh	N	J	P	Isopropanol	NBT, PMS	NAD	-	-
Ald	C2	C	U	Fructose-1, 6-diphosphate	NBT, PMS	NAD	GA-3-PD	-
Fum	O	O	R	Fumaric acid	NBT, PMS	NAD	Mdh	-
ATPase	F1	F	F1	ATP	(Ammonium sulfate)	-	-	Ca^{++},Pb^{++},Mg^{++},K^{+},Na^{+}

(*) Staining is performed by agar overlay.