Pla, C., J.B.Toral and A.Fontdevila. Universidad Autonoma, Bellaterra (Barcelona), Espana. Genetic analysis of five morphological mutants recovered from a natural population of Drosophila buzzatti.

On October 1981, more than 300 adults of Drosophila buzzatti were collected at Calablanca, a country farm located at the outskirts of Sitges, about 45 Km south of Barcelona (Spain). This collection was performed at a row of Opuntia ficus-indica stands either using banana traps or aspirating the adults directly from the cactus rots. Thirty inseminated females of this collection were placed individually in vials and each offspring was investigated for its hidden morphological variability following the method of Spencer (1947). Accordingly, eight sib-pairs were established from each isofemale F_1 progeny. The analyses of their F_2 offspring unveiled the presence of five recessive eye color mutants, although test crosses showed that two of them were alleles of the same locus. Chromosomal assignment of the four independent mutants was performed by conventional linkage analyses using the offspring of crosses with marked strains. The tester strains used were homozygous for the following allozyme markers: M12 (Estβ, chromosome 2); S (ADH, chromosome 3) and M16 (PGM, chromosome 4) (Pla et al. 1984). Results of this analysis and considerations of reported data on chromosomal and mutant homologies (Stone 1955; Linsley & Grell 1968; Zouros 1976) substantiate the following tentative names, symbols and chromosome locations for the analyzed mutants: vermilion - v (X); mahogany - ma (2); scarlet - st (4) and brown -bw (5).

These are the first morphological mutants ever described in D.buzzatti. The low frequency of mutants in the studied sample (only four mutants out of thirty isofemale lines) may be explained in terms of the dynamics of natural populations of D.buzzatti and other cactiphilic species. Spencer (1940, 1941, 1944) has found obvious differences in frequency of occurrence of visible mutations between two natural populations of D.immigrans and D.hydei. The different degree of genetic variability for each of both populations of these species has been interpreted in terms of the reduction of crossbreeding as a consequence of a sharp population reduction during winter periods, which produces bottlenecks every year. In our particular case, the population of Calablanca is maintained by the rotting fruits and pads of not more than a few dozens of O.ficus-indica pads. The abundance of these natural substrates is seasonal, being high when there is an adequate combination of temperature and humidity. These optimal conditions occur only at few occasions, as we know by our collecting experience during several years. Consequently, population size experiments dramatic bottlenecks followed by expansions, which results in a low effective population size. This increases the frequency of homozygous and the effect of homoselection, producing a low equilibrium mutation-selection for morphological characters.

References: Linsley,D.L. & E.H.Grell 1968, Carn.Inst.Wash.Publ. 627; Pla,C., J.B.Toral, H.Naveira & A.Fontdevila 1984, submitted to Experientia; Spencer, W.P. 1940, Ohio J.Sci. 40:345-361; ____ 1941, Ohio J.Sci. 41:190-200; ____ 1944, Genetics 29:520-536; ____ 1947, Avd.in Genet. 1:359-402; Stone,W.S. 1955, Symp.Quant.Biol. 20:256-270; Zouros,E. 1976, Genetics 83:169-179.

Poole, J.H. and L.K.Dixon. University of Colorado at Denver, Colorado USNA. Drosophila peroxidases: I. Three major isozymes observed.

The response of peroxidase (PO) activity to pH was measured in homogenates of Drosophila melanogaster, and the correlations among variant forms of PO was determined.

Preparation of extract. A water soluble extract of Drosophila homogenate was prepared as follows. Flies were killed by placing in the freezer in pre-chilled bottles (-15°C) for 9 minutes (this was found to be the minimum time sufficient to kill all flies). A sample of 200-500 imagoes of mixed age and sex was weighed, and homogenized in distilled water (0.100 ml/mg tissue) with a motorized teflon-glass rotary tissue grinder (20 pulses per extraction at 10 second intervals). Use of pre-chilled (2°C) water for the extraction was sufficient to prevent frictional heating of the homogenate above 35°C. Homogenate was centrifuged at 2400 rpm for 20 minutes. All assays were performed on the supernatant solution.

A series of stock buffers was prepared covering pH 4.0-11.0 in increments of 0.1 pH unit, using phosphate-tris-borate (30 mM each) in the range pH 5.5-11.0, and phosphate-tris-phthalate (30 mM each) in the range pH 4.0-6.0. Then 0.20 ml Drosophila extract was mixed with 0.60 ml of each buffer and the resultant pH measured. These samples were adjusted to each

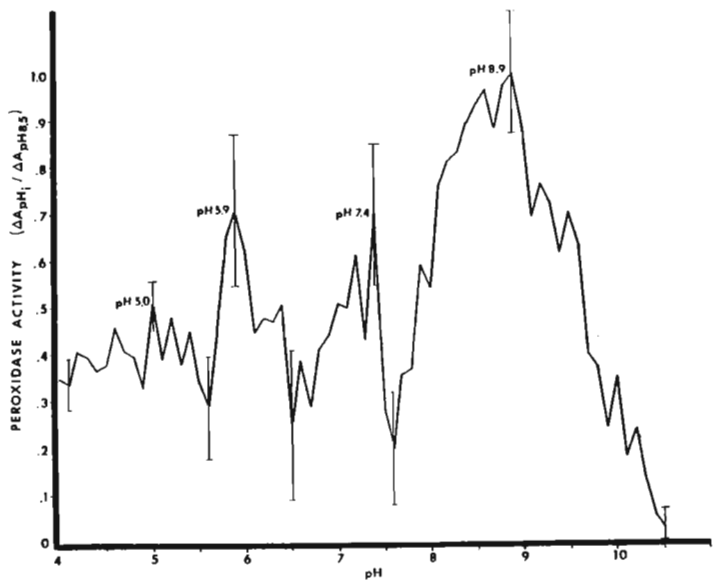

Fig. 1. Peroxidase activity vs. pH.

pH increment (±0.03) by blending samples of adjacent pH. In this manner, a series of samples was obtained for a given extract, spanning the desired pH range.

Assay method. To measure PO activity, the rate of oxidation of p-phenylenediamine (PDA) by hydrogen peroxide was photmetrically determined (Aurand et al. 1956; Armstrong et al. 1978). In all assays, 100 ul PDA was mixed with 100 ul H_2O_2 (final concentration of each substrate = 20 mM) at 20°C in a 1 cm light-path cuvet, and the reaction was initiated by the addition of 600 ul buffered Drosophila extract (total volume = 0.8 ml). Photon absorbance at 485 nm was measured for 10-20 minutes, and zeroed throughout the reaction against a blank cuvet. (The contents of the blank were the same as for the sample cuvet, but with plain buffer rather than extract.) The rate of change in A_{485} was used as a measure of the reaction velocity (i.e., formation of the reaction product, diaminophenazine). PO activity was determined from the mean slope in the linear portion of the reaction curve.

pH optima. A total of 580 measurements of PO activity were obtained between pH 4.0 and 10.5, at 0.1 pH increments (7-14 independent determinations per point). Raw activity scores were transformed to percent of the activity at pH 8.5, prior to calculation of the mean relative activity at each pH increment. This analysis resolved the presence of four activity optima, at pH 5.0, 5.9, 7.4 and 8.9 (Fig. 1). For each of these peaks the level of PO activity was significantly greater than that of the flanking minima (one-tail t-test), with $p<.02$, .02, .01 and .001 respectively. The three largest peaks displayed activities 3-5 times greater than the adjacent minima. These peaks will be referred to as acid-PO (pH 5.9), neutral-PO (pH 7.4) and alkaline-PO (pH 8.9). The fourth, small peak (pH 5.0) was not given further attention in this initial analysis.

Armstrong et al. (1978) also reported a pH optimum for Drosophila PO at pH 7.4. The other peaks reported here were not observed, perhaps due to the smaller number of measurements taken over wider pH increments in that study.

Evidence of isozymal status. Distinct pH optima generally correspond to variant forms of an enzyme (see IUPAC-IUB, 1976, for discussion). The question may be raised whether the three major peaks observed in this study correspond to distinct isozymes of PO. Another possibility is that only a single polypeptide is synthesized, which is subsequently modified by conjugation, cleavage or conformational changes (such alternate forms are not properly considered isozymes). If the latter alternative were correct, one would expect to find consistent correlations among the activities of each enzymatic form.

As a preliminary test of these alternatives, the activities of acid-PO, neutral-PO and alkaline-PO were measured in males of 12 inbred strains and a random-bred population of D. melanogaster (3-week-old imagoes). For each strain, four independent assays were performed at each pH, with 10 flies per assay. Across all strains, the following product-moment correlations were obtained among the activities of the PO variants (12 df):

$r_{\text{acid-PO neutral-PO}} = 0.35$ (N.S.)

$r_{\text{neutral-PO alkaline-PO}} = 0.52$ (N.S.)

$r_{\text{acid-PO alkaline-PO}} = 0.30$ (N.S.)

The absence of significant correlation (at the .05 level) strongly indicates that these PO variants are in fact independent isozymes. The distinct kinetic properties of the PO's (see the following paper in this series) provide further verification of the isozymal status of these variants.

It is of interest to note that the only electrophoretic analysis of insect peroxidases reported to date (Coles 1966) isolated three major PO isozymes in locusts. The three Drosophila PO's observed in the present study may well be homologous to the three locust isozymes. Electrophoretic analyses of the Drosophila peroxidases are currently in progress in our lab.

References: Armstrong,D., R.Rinehart, L.Dixon & D.Reigh 1978, Age 1:8-12; Aurand,L.W., W.M.Roberts & J.T.Cardwell 1956, J.Dairy Sci. 39:568-573; Coles,G.C. 1966, J.Insect Physiol.. 12:679-691; IUPAC-IUB Commission on Biochemical Nomenclature 1976, Handbook of Biochemistry and Molecular Biology, 3rd ed., V2, G.D.Fosman ed. CRC Press, Cleveland Ohio 1976 pp 84-84.

Poole, J.H. and L.K.Dixon. University of Colorado at Denver, Colorado USNA. Drosophila peroxidases: II. Isozyme kinetics, and optimum conditions for assays utilizing p-phenylenediamine.

The purpose of this study was to obtain initial estimates of the kinetic properties of Drosophila peroxidase (PO) isozymes, and to develop a sensitive photometric assay for each PO, for use on Drosophila tissue homogenates. A series of reactions was carried out to measure the effect of substrate concentration on reaction velocity, and to identify optimum assay conditions.

PROCEDURES: Flies were killed and extract prepared as described in the previous study in this series (see previous note). Subsequent to finding three major pH optima for PO activity (previous study), all extractions were carried out in tris-phosphate buffer (33.3 mM each) at pH 5.90, 7.50 or 9.05 (±0.01), 0.100 ml buffer/mg tissue. This yielded homogenates with the target pH of 5.9, 7.4 or 8.9 (±0.05).

PO activity was determined from the rate of oxidation of p-phenylenediamine (PDA) by hydrogen peroxide, measured as the change in absorbance at 485 nm (see previous note for general procedure). For each of the extracts (pH 5.9, 7.4, 8.9) a series of reactions was carried out with final concentration of H_2O_2 fixed at 20 mM, and the final concentration of PDA varied between 0.156 mM and 80 mM. This permitted estimation of the K_m for the PDA substrate. Following this determination, a series of reactions was carried out with the concentration of PDA fixed at 40 mM (i.e., greater than 75% enzyme saturation) and H_2O_2 concentration varied between 0.156 and 80 mM. This allowed estimation of the K_m for the H_2O_2 substrate.

KINETIC PROPERTIES OF THE PEROXIDASE ISOZYMES: Each of the three PO's produced linear Eadie-Hofstee plots (i.e., Michaelian kinetics) at all concentrations of PDA, and at concentrations of H_2O_2 below 5 mM. Catalatic activity was apparent as bubbling in the cuvet above 5 mM H_2O_2 --consistent with the observation of Angermueller and Fahimi (1981) that catalase activity predominates above 10 mM H_2O_2. The following estimates of K_m were obtained for each isozyme, based on the Eadie-Hofstee plots. For the H_2O_2 substrate, acid-PO had the highest apparent K_m=31±16.3 uM. Alkaline-PO's apparent K_m was 110±65 uM. For the PDA substrate, apparent K_m= 9±4.2 mM for acid-PO, 3.0±1.10 mM for neutral-PO, and 5.5±2.15 mM

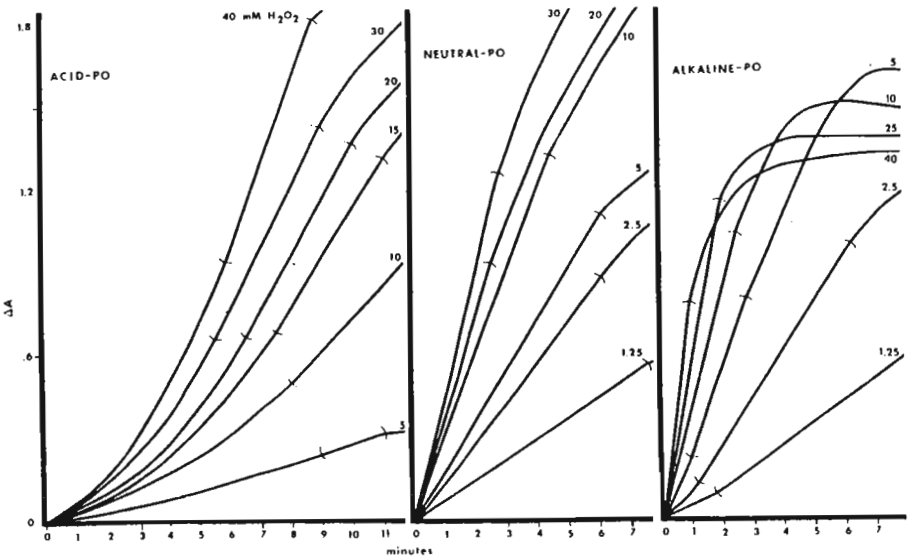

Fig. 1. Time course of the peroxidase reactions at various substrate concentrations. In all reactions the concentration of PDA was twice the concentration indicated for H_2O_2. Hysteretic lags were evident for acid-PO and alkaline-PO activity. Parentheses enclose the period of maximum linear velocity.