Poole, J.H. and L.K. Dixon. University of Colorado, Denver, USNA. Drosophila peroxidases: IV. Heritability (H) estimates for the three major isozymes.

In this report, we present our findings on quantitative variability of the three major peroxidase (PO) isozymes in **Drosophila melanogaster**, with estimates of the coefficient of genetic determination for each isozyme's activity. The study utilized 3-week old male adults from a wildtype population and 13 derived inbred strains.

METHODS: All stocks were maintained on cornmeal-molasses-agar-yeast medium in half-pint bottles (200-400 flies per bottle), with a diurnal cycle of 12 hr in the light at 25°C and 12 hr in the dark at 18°C. The wildtype sample was obtained from the National Center for Atmospheric Research, Boulder, Colorado (Dr. Edward Martell). Thirteen inbred strains were derived by 25 generations of full-sib mating. Stable populations of these strains were then built up by 2 months of free mating within each strain, during which all transfers and collections were carried out without the use of ether. Imagoes were collected at eclosure and transferred weekly to fresh medium, thereby maintaining distinct age-cohorts.

For assays, 3-week old (±3.5 days) imagoes were frozen, sexed, weighed, homogenized in buffer, centrifuged and photometrically assayed with PDA/H_2O_2 as previously described (Poole & Dixon 1984). Ten males were pooled for each extraction and assay. From each inbred strain and the wildtype population, four independent extractions and assays were performed for each of the PO isozymes (acid-PO, neutral-PO, alkaline-PO). PO activity is reported in picokatals (picomoles H_2O_2 reduced per second) at V_{max} per mg body mass (for conversion factors, see Poole & Dixon 1984). One-way analyses of variance were used to test the significance of inter-strain differences, for each isozyme's activity.

Heritability (H) was estimated from total and residual variances, by calculation of the adjusted coefficient of determination:

$$\text{(eq. 1)} \qquad H = \bar{R}^2 = 1 - (V_{resid}/V_{total}) \ ,$$

and the standard error of the coefficient was estimated as:

$$\text{(eq. 2)} \qquad S_{\bar{R}2} \simeq \left[2 \frac{V^2_{resid}\, a^2\, (b-1)(a+b-4)}{V^2_{total}\, b^2\, (a-3)^2\, (a-5)} \right]^{\frac{1}{2}} \ ,$$

where a=51 = total degrees of freedom, and b=39 = residual degrees of freedom (Cavalli-Sforza & Bodmer 1971).

Table 1. Peroxidase isozyme activities of inbred strains. Each data point is based on 49 males, age 3-weeks. ANOVA on inter-strain differences (inbreds only):
- acid-PO, $F(12,39) = 17.5$ ($p<0.0005$)
- neutral-PO, $F(12,39)=8.58$ ($p<0.0005$)
- alkaline-PO, $F(12,39)=7.17$ ($p<0.0005$)

	Peroxidase activity (pkat/mg tissue)					
	acid-PO		neut-PO		alk-PO	
Strain	mean	± SE	mean	± SE	mean	± SE
1	144	18.0	90.8	26.8	124	9.0
2	59	8.2	42.2	3.0	84	5.2
3	46	8.7	6.9	4.0	92	4.9
4	61	7.1	58.2	6.7	88	2.1
5	26	5.1	68.6	2.76	91	8.9
6	37	7.2	71.0	7.2	71	2.3
7	47	6.7	41.8	3.12	82	6.7
8	176	21.5	85.6	5.4	72	4.1
9	93	13.9	23.9	3.4	89	1.2
10	69	12.8	18.6	2.52	92	3.1
11	97	29.2	22.8	0.96	62	7.5
12	229	15.9	67.4	1.44	86	11.8
13	67	11.8	25.6	2.40	57	4.4
wildtype	96	7.2	77.5	5.4	72	1.6

RESULTS: Table 1 lists mean activities of the three PO isozymes in males of each strain. Large inter-strain differences were found for each isozyme. These differences are highly significant ($p<0.0005$ for each isozyme) and account for a large proportion of the overall observed variability in each isozyme's activity ($\eta^2=0.84$ for acid-PO, $\eta^2=0.73$ for neutral-PO, $\eta^2=0.69$ for alkaline-PO).

Table 2 lists Heritability (H) estimates for each isozyme's activity in males. The calculated value of H (eq. 1) is equivalent to $V_{between\ strains}/V_{total}$. If it is assumed that environmental variability is equally distributed over all strains and that there are no genotype-environment interactions, then H is an estimate of the proportion of variance in PO activity that is due to genetic variability in the overall population. It should be noted that H is a measure of heritability in the broad sense, which does not differentiate among additive, dominance and epistasis components of genetic determination. The results of this study indicate that in a heterogenous population, under standard laboratory conditions, about 60-80% of the variance in PO activity is under genetic control.

Heritable differences in the activity of a given PO isozyme may be due to any of the following types of genetic variability: allelic differences in the PO structural gene, allelic differences in PO regulatory

Table 2. Heritability estimates for peroxidase isozyme activities in males. H and its standard error were calculated using equations 1 and 2.

Isozyme	H
Acid-PO	0.79 ± 0.07
Neutral-PO	0.64 ± 0.12
Alkaline-PO	0.59 ± 0.13

loci (affecting quantity or developmental timing of PO synthesis), and allelic differences in substances that directly modulate PO enzyme function. We are currently engaged in a number of studies to examine these alternatives. First, if heritable differences in enzyme structure are present, such polymorphisms should be demonstrable by electrophoretic procedures we are applying to the Drosophila peroxidase system (viz., Lichtenstein et al. 1984). Second, segregation analyses on the offspring of inter-strain matings may allow us to differentiate the additive, dominance and epistasis components of PO heritability, and to estimate the total number of genetic loci involved in regulation of each PO isozyme's activity. Finally, we are engaged in a number of studies to determine the role of each PO isozyme in Drosophila metabolism--and the possible functional significance of heritable differences in each isozyme's activity.

References: Cavalli-Sforza, L.L. & W.F. Bodmer 1971, in:Genetics of Human Populations, Freeman & Co., San Francisco, p574; Lichtenstein, P.S., M. Emmett, L.K. Dixon & A.J. Crowle 1984, DIS 60:138-140; Poole, J.H. & L.K. Dixon 1984, DIS 60:165-168.

Ramachandra, N.B. and H.A. Ranganath.
University of Mysore, India. Further studies on B-chromosomes in **D.nasuta albomicana.**

Supernumerary or B-chromosomes have been reported in over 1000 plant species and 260 animal species (Jones & Rees 1982). In Drosophila, the occurrence of B-chromosomes is rare. The presence of such chromosomes were first detected by Kitagawa (pers. comm.) in **D.n.albomicana.** Recently we have reported the preliminary cytology of B-chromosomes in a Thailand strain of **D.n.albomicana** (Ramachandra & Ranganath 1984, 1985). After this preliminary screening that is in 1983, the Thailand strain was maintained under optimal conditions in the laboratory at 22°C for over two years and again the karyotypic composition of the individuals of this strain was analyzed. The important observations are as follows:

(a) Six different types of individuals with different number of chromosomes were recorded. They are without B's, with one, two, three, four or five B-chromosomes.

(b) Individuals with four and five supernumeraries were not recorded earlier. The meterphases with these B-chromosomes are presented in Figures 1a and 1b.

(c) The comparative account of the frequencies of different individuals with different B-chromosomes in the same strain of **D.n.albomicana** during 1983 and 1985 is given in Table 1. There is a significant decline in the incidence of individuals without B-chromosomes. The frequency of

Table 1. Relative frequencies (%) of individuals with different number of B-chromosomes in **D.nasuta albomicana** during 1983 and 1985 under laboratory conditions.

Years	0B	+1B	+2B	+3B	+4B	+5B
1983	33	36	26	05	--	--
1985	05	32	38	21	03	01

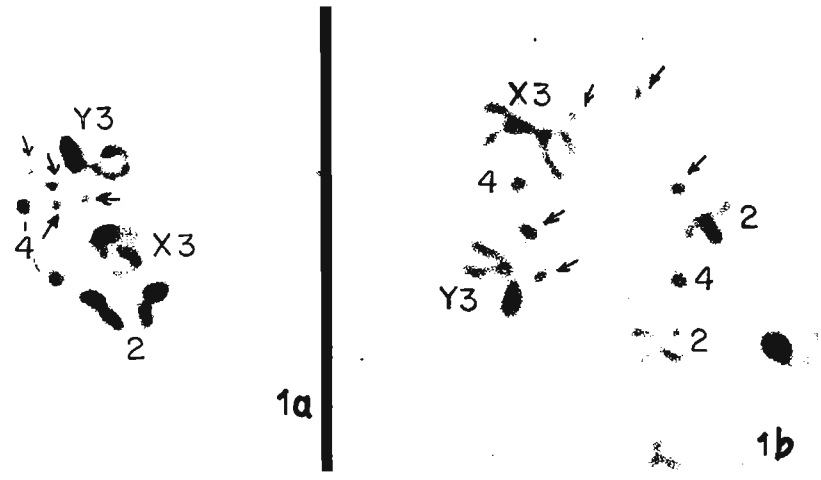

Figure 1a-1b: Karyotype of **D.n.albomicana** with 4 (1a) and 5 (1b) B-chromosomes. Arrows indicate B-chromosomes.