the histogram (index of heterogeneity) according to the method of Lalande and Miller[24]. These indices, together with the total number of cells required to obtain 4000 cells with mean fluorescence intensity corresponding to the modal intensity, reflect the measure of heterogeneity of cell lines.

ATC-10 and ATC-121 cell lines thus appear to be relatively less heterogeneous because these contained relatively fewer cells beyond the modal population and also because these required a lesser number of cells to generate a histogram with 4000 cells in the peak frequency channel. The histograms are a composite profile of cells in G_1, S and G_2+M phases of the cell cycle. In such a case, the larger the heterogeneity in the cell population, the more skewed is the fluorescence distribution at higher intensities. The widespread distribution of DNA values obtained for Feulgen cytophotometric estimation in the 2 lines tested also indicates the heterogeneity of these cell lines. It thus appears that data on DNA estimations by either method are comparable to those from chromosome studies.

The diploid cell lines maintained in continuous cultivation - subcultured after reaching confluency - have a finite lifespan. However, from the data presented here, it appears that the established mosquito cell lines, which have traversed more than 100 passages, are karyologically heterogeneous and contrary to all the earlier reports are shown to be not diploid. Further, it may be mentioned that of the 3 cell lines, viz., ATC-10, ATC-15 and ATC-121, the 1st 2 could induce neo-vascularization in chick chorioallantoic membrane, indicating the presence in these of an angiogenic factor; however, when inoculated in conditioned golden hamsters and albino mice, none of the 5 cell lines produced tumors[25]. Studies on agglutinability of cells after concanavalin-A treatment revealed presence of 2 cell populations in late passage mosquito cell lines: one agglutinable and the other non-agglutinable, indicating the occurrence of transformed as well as untransformed cells[26]. These findings suggest that the established mosquito cell lines exhibit characters which are generally not shown by diploid cells and thus lends support to the observation that these cell lines are not diploid.

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Enzymes involved in oxygen detoxification during development of *Drosophila melanogaster*[1]

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Summary. Activities of superoxide dismutase (SOD), catalase (CAT), and peroxidases (PER) were examined at 24-h intervals during *Drosophila* devolopment. SOD activities show a U-shaped curve from egg to adult stages whereas CAT and PER are consistently low in egg through larval stages. Male and female larvae have similar activities of SOD, CAT, and PER whereas male adults have elevated activities of these enzymes. Larvae are more sensitive to H_2O_2 and 3-amino-1,2,4-triazole (an inhibitor of CAT) than adults.

The toxicity of oxygen in living systems involves direct oxidation of thiol groups of enzymes, and production of toxic intermediates such as hydrogen peroxide, hydroxyl radical or metal oxy-compounds generated by superoxide-mediated Fenton chemistry[2,3]. The tripeptide glutathione beneficially intercedes in enzyme oxidation, while peroxidases (PER) and catalase (CAT) are effective in maintaining relatively low levels of H_2O_2. Superoxide dismutase (SOD) converts the superoxide anion in a disproportionation reaction to H_2O_2 and O_2 and may indirectly prevent the formation of the reactive ferryl ion (complex FeO_2^+) and/or an organic oxyradical $RO\cdot$[4]. Under atmospheric oxygen concentration, these defense systems protect organisms from obvious stress. In hyperoxia, these systems appear to be 'swamped' and a toxic syndrome is observed[5,6].

We recently reported on the influence of increased O_2 during development of *Drosophila melanogaster*[7]. We observed that 1st, 2nd and early 3rd instar larvae are extreme-

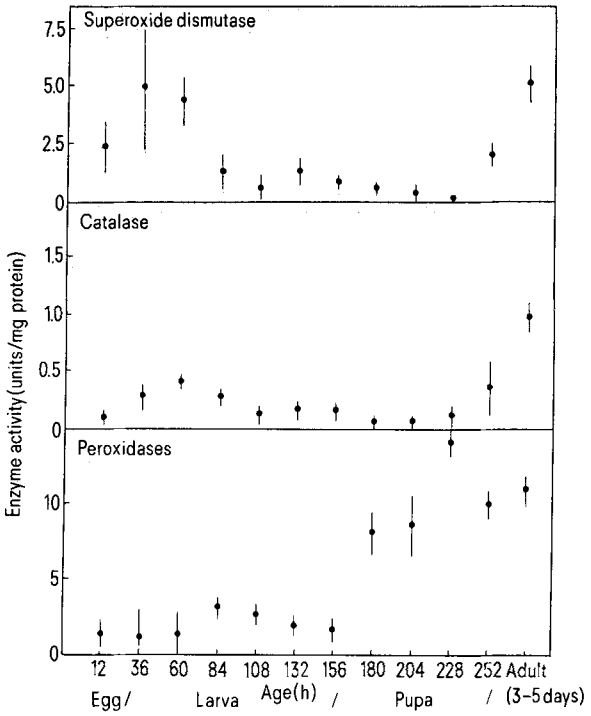

Figure 1. Activity in units/mg protein of SOD, CAT, and PER during *Drosophila* development. Each mean consists of 2 readings. Vertical lines indicate SE.

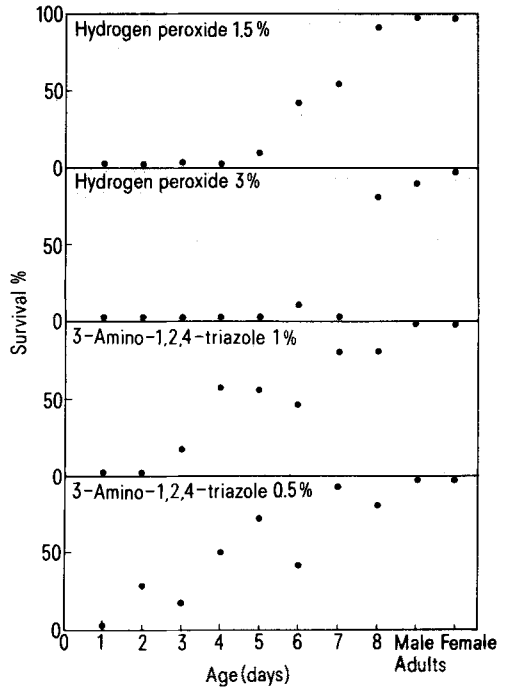

Figure 2. Percent survivorship of *Drosophila* exposed for 4 h during various stages of development. Expressed as percent of control (Ringers treated). Each point represents a mean of 2 experiments with 20 organisms at each developmental stage.

ly sensitive to elevated O_2 (normobaric) whereas eggs, late 3rd instar larvae and pupae are relatively insensitive under our exposure conditions. Eclosing flies exposed to an O_2-enriched environment as pupae consistently possessed eye abnormalities. The work reported here describes the activities of SOD, CAT, and PER at successive 24-h periods during *Drosophila* development. In addition, we report sex dimorphism, and sensitivities to elevated levels of H_2O_2.

The Urbana strain of *Drosophila* was used with 1st, 2nd, and 3rd chromosomes rendered 'isogenic' by use of the following balancer chromosomes: CyO/S Sp Bl L^{rm} bw^D, $In(3LR)D_{cx}F$ ru h D ca/kar^2 D, Binscy/C(1)Dx, yf; Muller 5, Pm, Sb, Ubx^8. Fertilized eggs were collected from 3 to 5 days old females at 24-h intervals from yeast-soaked media surfaces. They were placed 50-each in shell vials containing 5 cm^3 of standard cornmeal, molasses, sucrose, agar medium. Each enzyme assay or treatment (H_2O_2) was conducted in one time period on organisms of all developmental stages. Material was washed in insect Ringers and homogenized in 3 ml of 0.15 M phosphate buffer, pH 7.4[9]. Each homogenate was cleared by centrifugation at 15,000 rpm for 15 min at 4 °C. CAT was assayed by the method of Luck[10] using 0.05 ml of homogenate. Activity of SOD was determined using the method of Rapp[11] using 0.2 ml of homogenate. One SOD activity unit was defined as causing a 50% reduction in absorbance at 580 nm. No attempt was made to distinguish between cytoplasmic and mitochondrial forms of SOD. The method of Armstrong[9] was used to assay PER on 0.6 ml of homogenate. Protein concentration was determined by the method of Lowry et al.[12]. The sensitivity of H_2O_2 or 3-amino-1,2,4-triazole (AT) was determined by placing a constant number of washed specimens in various concentrations of these materials (in Ringers) for a 4-h interval.

As shown in figure 1, 3 distinct profiles exist for activities of SOD, CAT, and PER. SOD levels are relatively high in eggs and young larvae, but decline significantly as larvae age. Old pupae and adults show relatively high levels of SOD. These results are in agreement with those of Massie et al.[13], however they were unable to note the relatively high levels of SOD in eggs and early 1st instar larvae. In addition, our results do not reflect as dramatic of change in SOD activity during metamorphosis. This may be due in part to different strains used and/or the fact that they compared their activities to mg wet weight while ours were compared to mg protein. Eggs of the Mediterranean fruit fly, *Ceratitis capitata*, as well as preadult stages contain low and fairly constant SOD levels when compared to adults[14]. Activity of CAT is barely detectable in eggs and generally low throughout larval life. Our results are in general agreement with those of Baird et al.[15] for the Oregon-R strain, however we found the increase in CAT activity to occur somewhat later in the pupal stages (late pupae rather than mid pupae). PER has a dramatic surge in activity in prepupal stages after which activity drops in the adult stage. These results are similar to those of Armstrong[9] who examined PER levels throughout late larval, pupal, and adult stages.

In the table we present activity levels of SOD, CAT, and PER in male and female 3rd instar larvae and 3-5 days adults. As expected from the discussion above, activities of

Comparison of mean activity of SOD, CAT, and PER in 3rd instar male and female *Drosophila* larvae and 3-5 days old male and female adults. Each mean is composed of 3 measurements

Enzyme	Mean activity (units/mg protein)				
	Male larvae	Female larvae	Male adults	Female adults	F-ratio
SOD	0.74	1.56	4.05*	1.28	17.57
CAT	1.43	2.50	8.00*	2.51	23.59
PER	1.08	2.46	7.20*	4.65*	127.15

*Indicates a significant difference at least of $p < 0.05$.

enzymes in adults are higher than in larvae. Sex of larvae has little if any significant influence on enzyme activities whereas male adults consistently maintain higher enzyme activities as compared to females. Armstrong et al.[9] also noted sex dimorphism in activity of PER in adult flies with the greatest difference being observed in the 21–26 days age group.

We examined the sensitivity of developing *Drosophila* to H_2O_2 and AT (a noncompetitive inhibitor of CAT resulting in the irreversible destruction of CAT without interferring with the rate of new synthesis). Results of these studies are presented in figure 2. In each case (1.5%, 3% H_2O_2 1%, 0.5% AT) younger organisms are more sensitive to H_2O_2 or AT than older ones. These results are similar to those of Lubinsky and Bewley[16] who found 1st instar larvae most sensitive to AT (LD_{50} of 0.65 mM) and adults capable of injesting a solution of up to 10 mM for 8 days. The sensitivity pattern for H_2O_2 or AT does not follow that of O_2[7] perhaps reflecting different modes of toxicity. In addition, survivors of the H_2O_2 or AT treatment were free of the developmental abnormalities noted for the eyes of O_2-treated pupae. Because our preliminary studies (unpublished) included a range of H_2O_2 concentrations up to 30% in which no eye abnormalities were observed, we conclude that elevated levels of O_2 specifically alter eye pigmentation in *Drosophila.* Attempts to subject developing *Drosophila* to elevated levels of the superoxide anion failed due to toxicity of components of the generating system.

The relationship of SOD, CAT, and PER to O_2-provoked toxicity is uncertain. The results reported here show that increased sensitivity to O_2 and H_2O_2 is greatest when activities of SOD, CAT, and PER are relatively low. However, in our study and others[9], adult males have significantly higher levels of these 3 enzymes per mg protein than females, yet Kloek[5] found adult males more susceptable than females to elevated O_2. Age related changes in O_2 sensitivity and activity levels of SOD, CAT, and PER have been observed in other adult systems[5,6] and are suspected as being related to natural senescence and aging phenomena[17].

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Evidence for post-zygotic lag in *Chlamydomonas moewusii* (Chlorophyta; Volvocales)

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Summary. A wild-type characteristic may be transmitted through heterozygotes and manifest itself initially in haploid mutant progeny. Evidence for this is adduced from experiments with a paralyzed pf^- mutant of *Chlamydomonas.*

Although ordinarily the phenotype of cells, and hence of tissues, organs, plants or animals, is an expression of their nuclear genotype, this is not always so. A newly mutated gene, for instance, does not immediately manifest its presence: if it arose in a wild-type cell, then for a while – often for several cell generations – the phenotypic effects of its antecedent wild-type allele continue to be manifest. This phenomenon, as observed in various micro-organisms[2–4], has been called phenomic lag.

Another circumstance where phenotype may not immediately reflect genotype is in cells immediately following meiosis. A heterozygous diploid cell, with the phenotype of a specified dominant gene, typically cleaves into 4 meiotic products of which 2 bear nuclei with the dominant allele and 2 bear nuclei with the recessive allele; but all 4 inherit from the zygote cytoplasmic factors which had been produced by the dominant gene. In microbial genetics the phenotype is not usually examined until after several post-meiotic divisions have given rise to colonies or clones, by which time such residual products of parental dominant genes have been diluted out or otherwise caused to disappear. However, in some cases even individual post-meiotic cells can be seen to manifest certain genetic characteristics. This is true, for instance, for motility of algal flagellates such as *Chlamydomonas.*

We present here evidence for what we propose to call post-zygotic lag, in which certain vegetative (haploid) cells of *C. moewusii* known to carry a gene for flagellar paralysis (pf^-) nevertheless can swim normally. Germinating zygotes from a cross $mt^+ \cdot pf^-$ (mutant strain M.1002)[5] $\times$ $mt^- \cdot pf^+$ (wild type) give rise initially to 4 haploid cells which can be grown to produce sub-cultures of 2 kinds, with cells respectively motile (pf^+) and paralyzed (pf^-), in equal numbers (2:2 segregation). However examination of the behavior of those first 4 meiotic products reveals that they all can swim normally. One simply immerses germinating zygotes singly