This approach allows the determination of the relative volume of the serotonin-containing granules and the equilibrium coefficients for AO transport across the plasma and granule membranes. 8 µl of AO were injected into three PRP samples 0.8 ml each (final concentrations were 2, 4 and 8 µM). The samples were incubated for 40 min and AO fluorescence intensities were measured. Fluorescence intensities of corresponding AO concentrations in autologous PPP were also measured to take into account the probe fluorescence changes in plasma compared to its fluorescence in normal saline. The following formula was used for this correction:

$$F_0 = f_0 \times f(\text{saline})/f(\text{ppp}),$$

where f (saline) and f (ppp), AO fluorescence in normal saline and PPP, respectively; f_0, AO fluorescence in PRP. The calculated values F_0 for different concentrations of AO were substituted into three equations type (2), and the set was solved with a computer.

The following results were obtained for healthy donors (n = 5) and rabbits (n = 10):

human platelets $\rightarrow W = 0.14 \pm 0.01$,
$K_1 = 375 \pm 60$, $K_2 = 2260 \pm 382$;

rabbit platelets $\rightarrow W = 0.29 \pm 0.02$,
$K_1 = 225 \pm 60$, $K_2 = 30\,000 \pm 550$.

So far, the granule apparatus has been characterized by the size (average diameter) and the number of granules determined by electron microscopy[17–19]. However, application of these parameters for characterization of the granule apparatus is complicated by the fact that they do not obey the law of normal distribution[20]. It was suggested that the relative volume of granules obeys the normal distribution law and is a more convenient criterion for characterization of the granule apparatus on accumulation and release. The results obtained confirm this suggestion and demonstrate an origin-specifity of the granule apparatus. In human platelets the relative volume of the granules was 14±2%. In rabbit platelets it was 29±2%, which is in good agreement with the results obtained by Pletscher et al. (30%) using differential centrifugation[21].

Rabbit platelets have the highest content of serotonin compared to that in human and guinea pig platelets[1]. Not surprisingly, both W and K_2 (the intragranular/extracellular AO concentration ratio) are greater in rabbit than in human platelets. Our data also indicate that the plasma membrane (parameter K_1) plays an important role in the process of accumulation.

It should be mentioned that the results are valid only for the assumption that F (C) (relationship between fluorescence intensity and probe concentration) is the same in the extracellular, cytoplasmic and intragranular compartments. This assumption was used since it is difficult to develop a model of intragranular space in vitro. Based on good agreement between our results and those reported by others, we have concluded that this assumption does not lead to considerable errors. It is noteworthy that in biomedical investigations not absolute values but the relative changes of the above mentioned parameters may be important.

The proposed method is simple and provides detailed information on accumulation of various substances in a vast array of cells. This approach can be used for the investigation of intestine and adrenal medulla chromaffin cells, synaptosomes, mast cells, and other cells capable of accumulation and storage of biologically active substances.

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The sensitive period for yellow phenocopy induction in *Drosophila melanogaster*

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Summary. Yellow phenocopies of *Drosophila melanogaster* were produced by raising larvae on α-DMT contaminated media. Using a survivorship test, the sensitive period for phenocopy induction was found to occur during the third larval instar of development, with increased survivorship at 1% α-DMT compared with lower concentrations. It was also found that treatment with α-DMT significantly slowed development. These findings are related to the relevant morphological and behavioral developmental pathways and to phenocopy induction.
Key words. Sensitive period; phenocopy; yellow; survivorship; *Drosophila melanogaster*.

The yellow mutant of *Drosophila melanogaster* represents one of the few documented cases of a genetic influence on behavior [3]. In addition, the yellow phenotype is a rare example where both behavioral and morphological developmental pathways are simultaneously perturbed [3, 4]. As well as an over-sclerotized cuticle, yellow males show an altered mating behavior, compared with wild-type males [3, 5, 6]. Recently, the yellow locus itself has been studied in depth, revealing the genetic basis for yellow cuticular coloration [7, 8]. Debate remains, however, over the origin of the yellow males distinct mating behavior whether it has a physiological and/or neurological basis [6, 9].

Equivalent yellow forms of *Drosophila melanogaster* can be environmentally induced via treatment with α-DMT (α-dimethyltyrosine) contaminated media [10]. Such induced forms that resemble genetic mutants are called phenocopies [11] and provide an important opportunity to investigate organism/environment interactions [12]. Such interactions take place through specific temporal 'windows' in development called sensitive or critical periods [13]. Although sensitive periods are known to be intimately involved in development, there is much debate as to their origins. There have been a number of explanations of the origins of sensitive periods centering on such issues as developmental complexity [14], assimilation [15] and the nature/nurture debate [13].

The aim of this study was to characterize the sensitive period for the yellow phenocopy (y_p) relating it to the appropriate behavioral and morphological developmental pathways. Indicators of the presence of both behavioral and morphological sensitive periods include measures of expressivity, penetrance and mortality. Characteristically, expressivity and penetrance increase while mortality decreases at the sensitive period.

Materials and methods. The strain of *Drosophila melanogaster* used in this study was bred from over two hundred females which were caught at Henderson, Auckland in July, 1985. From the wild-caught flies, a stock of over ten thousand flies was cultured in a population chamber [16] at low densities to ensure uniformity in quality of condition. From this stock, adults were placed on petri dishes containing normal media and left in the dark for the females to oviposit. After 2 h the adult flies were removed. At varying stages of development (1st instar [36 h], 2nd instar [60 h], 3rd instar [84 h]) the larvae were transferred from the petri dishes to small bottles containing 10 ml of media with the inducing agent, α-DMT at concentrations of 0.1%, 0.2%, 0.5% and 1.0%.

Larvae were placed on 25-mm^2 pieces of damp black paper. The pieces of paper were placed larvae-side-down into separate bottles. 24 h later the paper was removed from the bottles and the number of dead larvae on each was counted. The number of individuals which emerged was recorded each day and their condition described.

Results and discussion. As with genetic yellow, the induced y_p had a more yellow cuticle than wild-type individuals. Moreover, consistent with the description of McDonald [17], y_p had a less intense cuticular coloration than genetically yellow individuals.

The penetrance of the yellow condition was 100%; i.e., individuals exposed to 0.1 – 1.0% α-DMT all exhibited the yellow condition. Expressivity was uniform throughout all concentrations and larval instars. Due to the uniformity of the expression and penetrance of the yellow condition, only mortality could be used as an indicator of the sensitive period.

To test for a treatment effect across the three instars of the α-DMT, a χ^2multiway contingency table was constructed from the raw frequency data (numbers transferred against total number of emerged adults). Subsequent analyses showed all the above tests for homogeneity were significant (see table). It is clear that the given concentrations of the α-DMT resulted in different levels of survivorship among the three instars. (These statistics were calculated using the SAS package [18].) Therefore, different concentrations of α-DMT have detectable effects on each of the three instars.

Results of a two-way ANOVA from a test of homogeneity using survivorship of *Drosophila melanogaster* as the dependent variable to detect differences among instars and concentration of α-DMT.

	df	χ^2value
Instar	2	44.76*
Conc. of α-DMT	4	66.05*
Instar * Conc. of α-DMT	8	95.53*
Replicate (Instar * Conc. of α-DMT)	30	107.77*

* = significant at 0.01% level.

Figure 1 shows the varying effects of α-DMT on the survivorship of first, second and third instar larvae. Third instar larvae survived best, with the tolerance level actually increasing at the highest treatment level (1% α-DMT). These individuals exhibited a constant level of survivorship throughout the concentration range of α-DMT, whereas 1st and 2nd instar individuals showed a relative drop in survivorship at the 1% concentration level, as found by Burnet et al. [10]. In fact, the highest level of survivorship overall was shown by 3rd instar individuals treated with 1% α-DMT (the highest concentration used in the experiment). The extraordinary low survivorship exhibited by 1st instar individuals at 0.1% α-DMT (fig. 1) was due to fungal growth in one of the replicates.

During the 3rd larval instar, higher concentration levels of the inducing agent (α-DMT) were assimilated into the cuticle and therefore were tolerated with lower levels of mortality. In comparison, very high levels of mortality resulted from high dosage concentrations, outside this sensitive period or 'window' in development. For example, 1st instar individuals exhibited 95% mortality at 1.0% α-DMT.

In order to illustrate any possible influence of the phenocopy inducing agent on the ontogeny of the organism, the percentage emergence through time of the different instars was compared (figs 2–4). These results indicate that, especially in the 3rd instar, higher concentrations of α-DMT slowed development with more individuals emerging later at higher concentrations. The effect of α-DMT on *Drosophila melanogaster* was also manifested through temporal changes in development. Similarly, Burnet et al. [10] found that a feature of y_p induction was that development was slowed by exposure to α-DMT.

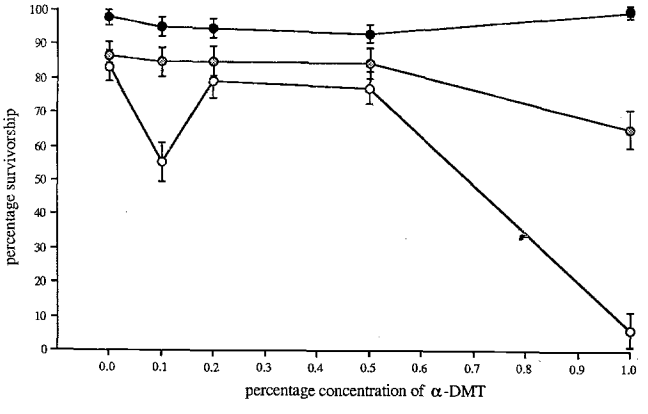

Figure 1. Survivorship of 1st (white), 2nd (grey) and 3rd (black) instar larvae at varying concentrations of α-DMT.

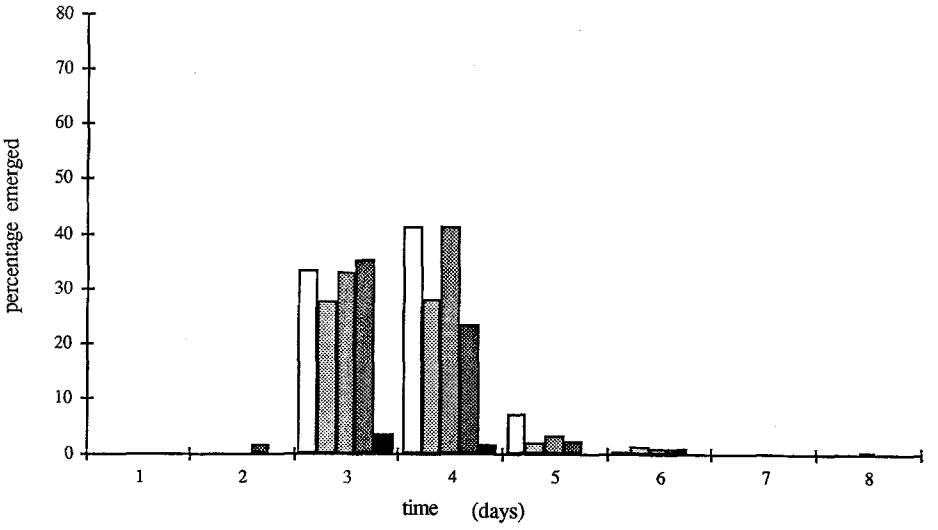

Figure 2. Percentage emergence through time of 1st instar larvae at 0% (white), 0.1% (light gray), 0.2% (gray), 0.5% (dark gray) and 1.0% (black) α-DMT.

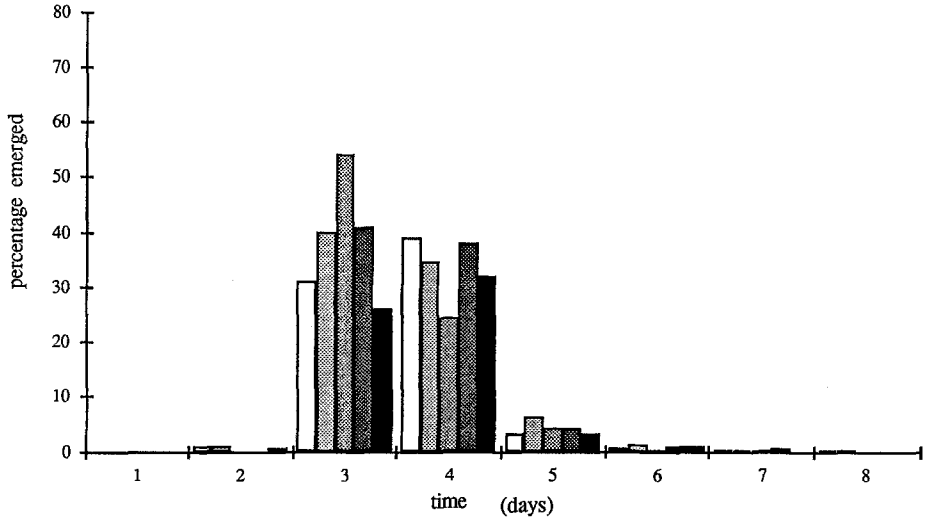

Figure 3. Percentage emergence through time of 2nd instar larvae at 0% (white), 0.1% (light gray), 0.2% (gray), 0.5% (dark gray) and 1.0% (black) α-DMT.

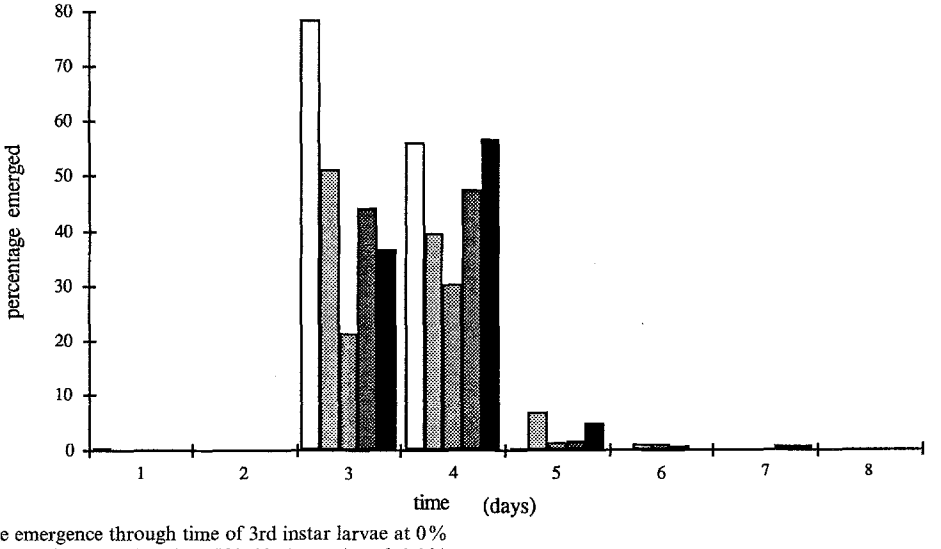

Figure 4. Percentage emergence through time of 3rd instar larvae at 0% (white), 0.1% (light gray), 0.2% (gray), 0.5% (dark gray) and 1.0% (black) α-DMT.

As in this study, the sensitive period for phenocopy induction is generally found to occur at an earlier stage of development than the expression of the equivalent genetic form. For example, the sensitive period for the induction of the bithorax phenocopy is only 2–2.5 h after ovipositing (the preblastoderm stage), compared with the formation of the wing imaginal disc which occurs during the 3rd instar stage [19, 20]. The results presented here illustrate that for y_p, the sensitive period precedes the major peak of both yellow mRNA expression, which occurs at the late pupal stage [7, 8 21] and the associated process, melanization, which occurs during day 4 of pupal development [8, 22]. Therefore, the sensitive period for the induction of the phenocopy occurs well before any of the known associated processes are initiated. Hence α-DMT is probably not *directly* affecting gene expression/action, or its associated developmental process, as expected by Goldschmidt [23] and Scott [14] respectively.

A possible route of induction of a yellow cuticle might be by a build up in the hemolymph of tyrosine residues which are subsequently converted to dopa [24], the main precursor of the melanization/sclerotization pathways [24, 25]. Since the degree of sclerotization is dependent on the amount of sclerotizing agent [26], phenocopy induction through the ingestion of the excess tyrosine in the form of α-DMT may be expected.

Because α-DMT induces a change in mating behavior [9, 10], as well as the cuticle of *Drosophila melanogaster*, the 3rd instar is also a sensitive period in the development of the Specific-Mate Recognition System (SMRS) [27, 28]. One possibility is that sexual behavior is being directly affected by α-DMT. Burnet and Wilson [6], for example, suggested that expression of the mutant yellow gene in the sexual foci residing in the thoracic ganglion may result in the impairment of mating ability. The chemical agent α-DMT might be affecting the sexual foci in a similar way. The sexual focus in the thoracic ganglion comprises one possible structure that interacts with α-DMT resulting in a divergent SMRS. Other possible structures, not related to the development of sexuality, that may be affected through the same third instar sensitive period, are the nervous system and the cuticle of the *Drosophila* itself [9].

If the sensitive period tends to occur prior to the expression of the associated gene or gene-complex, it seems unlikely that phenocopy induction is simply the 'suppressing, retarding or disorienting effect on one or more gene-controlling components of the normal or mutant genotype' [29]. In fact induction can occur via a number of different developmental pathways. Not only can the workings of the genetic nexus be directly affected by the inductive agent, as suggested by Blanc and Child [30] but, for example, in the case of y_p, induction may occur through increasing the amount of substrate (tyrosine) in the associated melanization and sclerotization pathways.

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Density of flies and male-crowding affect the outcome of interspecific crosses between *Drosophila simulans* and *D. mauritiana* and the hybrid progeny numbers

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Summary. When *Drosophila simulans* females from the Seychelles are crossed with *D. mauritiana* males the number of hybrids produced decreases with an increase in both density of flies and male/female proportion. The patterns are consistent in the reciprocal cross although the number of progeny is reduced. The asymmetry of mating success affects not only mating preference and the outcome of the cross, but also the progeny numbers.
Key words. Hybridization; asymmetrical mating success; male/female proportion; hybrid progeny number; *Drosophila mauritiana; Drosophila simulans.*