

# Esterase isoenzyme analysis of four ontogenetic stages of *Drosophila melanogaster* populations selected for fast and slow developmental time and treated with vertebrate and plant steroids

Ivana Beatrice Mânica Da Cruz and Alice Kalisz de Oliveira

## ABSTRACT

*Drosophila melanogaster* populations selected for 27 years for fast and slow developmental rate were treated with steroid hormones (ecdysone, vitamin D3 and *Solanum malacoxylon* (Solanaceae, Tubiflorae) vitamin D3-glycoside) to determine their effect on the esterase patterns of four ontogenetic stages (end of third larval instar, early pupae, pharate pupae and imagoes). Vitamin D3 promoted the greatest difference in esterase activity pattern. The effect of *S. malacoxylon* glycoside was most similar to that of ecdysone in the fast population and most similar to that of vitamin D3 in the slow population. When the similarity index was used to compare populations it was found that the *S. malacoxylon* treatment was the most effective in promoting differences in the esterase pattern between fast and control populations and the vitamin D3 treatment was the most effective in enhancing the difference between the fast and slow populations. In the comparison between slow and control populations, except for vitamin D3, all treatments including the untreated sample showed similar degrees of difference. Plant steroids differ from vertebrate hormone by an additional glycoside. Their effects on *D. melanogaster* metabolism differed and were dependent on endogenous hormonal content of the selected populations, which were previously found to be different (Jung, 1992).

## INTRODUCTION

To achieve coordinated developmental differentiation hormones must reach their target tissues at the appropriate time and with precise intensity. These requirements are met by precise regulation of effective titer, as has been found for *Drosophila melanogaster* development (Ashburner *et al.*, 1974). In this organism larva-to-adult metamorphosis is initiated by a pulse of the steroid hormone 20-OH-ecdysone (hereafter

referred to as ecdysone only), that occurs at the end of the third larval instar. This hormone pulse triggers a coordinated change in the developmental pathway of imaginal tissues that yield the adult structures and at the same time histolyzes most of the larval tissues.

Developmental rate is a fitness component important in ontogenetic regulation. Selection for fast and slow developmental rate carried out in our laboratory has produced *D. melanogaster* populations with special genomic sets, with consequent differences in puff activation size, which has led Loreto *et al.* (1988) to suggest hormonal influence since cytogenetic

analysis of late pupae showed lower activation in the slow population than in fast and control populations. The suggestion of hormonal imbalance was corroborated by Da Cruz (personal communication) using ELISA to measure the ecdysone content in zero hour pupae. Different patterns of gene activation chronology ( $r = 0.726$ ) and of general staining intensity of protein bands (total proteins, esterases, alkaline phosphatases, alcohol dehydrogenases, octanol dehydrogenases, leucineaminopeptidases, acid phosphatases, isocitrate dehydrogenase NADP dependent) were also found in pre-imaginal and imaginal stages when these populations were temporally compared (Oliveira and Cordeiro, 1982a,b, 1984; Loreto and Oliveira, 1988). The esterase isoenzyme patterns studied in eggs, first, second and third instar (0 to 55 h) larvae, white pupae, pupae with developed adult eyes, pharate pupae, one- and seven-day old adult males were different for fast and slow populations (Oliveira and Cordeiro, 1982b). Effects on pre-imaginal viability and developmental rate, abnormal spiracle eversion patterns and defective pupal development were also found with vertebrate vitamin D3 and *Solanum malacoxylon* treatments (Da Cruz *et al.*, unpublished results). This plant contains 1,25-dihydroxychole-calciferol-glycoside (Wasserman *et al.*, 1976) which, when ingested by cattle, causes a disease known as enzootic calcinosis (Wasserman *et al.*, 1976; Dobereiner *et al.*, 1972) which is characterized by a generalized mineralization of soft tissues due to calcium deposition in the cellular matrix of the arterial wall (Barros *et al.*, 1981). Aspects of cellular differentiation and modification at the extracellular matrix level appear to be intimately linked, demonstrating atherosclerosis-like action of the steroid hormone 1,25-dihydroxyvitamin D3. Using different hormonal treatments, Jung (1992) corroborated suggestions by Laudet *et al.* (1992) about a relationship between ecdysone and vitamin D3 receptors and by Pablo and Roth (1990) about a similarity between vertebrate and invertebrate molecules, suggesting that vitamin D3 and plant steroids analogs could affect the development of *D. melanogaster*.

The effect of these steroids on isoenzymatic esterase patterns of four ontogenetic stages of *D. melanogaster* selected or not for developmental time was analyzed.

## MATERIAL AND METHODS

Oregon-R populations of *D. melanogaster*, selected during 650 and 433 generations for fast and

slow developmental rate, respectively, and a control population were used (Oliveira and Cordeiro, 1981). They were named F = fast population, S = slow population and C = control population.

### Steroid treatments

Three hundred fly pairs, aged four-five days, from the three populations, were placed on oviposition medium for one hour. One day after oviposition the first instar larvae were treated with: 1) ecdysone (Sigma,  $1.5 \times 10^{-6}$  M), as a negative control. This concentration was used by Ashburner *et al.* (1974) for *in vitro* salivary gland treatment and, although low, was used here to standardize the hormone level of the populations and for comparison with other treatments. 2) Vitamin D3 (Sigma) at a concentration of 50,000 units/ml, the concentration (in a dose of 1 ml) usually given to patients with vitamin D3 hormonal insufficiency; 3) 2 g/100 ml *S. malacoxylon* extract, prepared as suggested by Barros *et al.* (1981), and 4) nothing, as a positive control. Treatments were made by oral administration, because steroids penetrate the cuticle less readily than the smaller juvenile-hormone molecules (Robins, 1972).

For the esterase isoenzyme pattern the samples were collected at four ontogenetic stages: larvae at the end of the third instar, pupae with adult eyes developed (here named Pupa 1); pharate pupae (Pupa 2) and imagoes. Twenty-four individuals were analyzed per treatment.

### Gel electrophoresis

Isoenzyme patterns were studied in polyacrylamide gel electrophoresis (6% Cyanogum) using the discontinuous buffer system described by Poulick (1957): 0.076 M Tris-citrate buffer, pH 8.65, containing 0.005 M citric acid. The bridge buffer was borate, pH 8.6 (0.34 M boric acid and 0.05 M sodium hydroxide). This electrophoresis was performed at 0°C. An electric field of 10 V/cm was applied across the gel for three to four hours, or until the migrating front was nine cm from the sample slot line. To develop esterase zymograms, the gel was incubated in phosphate buffer 0.01 M, pH 6.0, for 20 min, and for approximately one hour and 30 min at 38°C in the following staining mixture: 100 ml 0.01 M phosphate buffer, pH 6.0; 100 mg Fast Blue BB; 1.5 ml 1% alpha-naphthyl acetate and 2 ml 1% beta-naphthyl acetate (substrate diluted in acetone and water, 1:1).

The data were analyzed statistically by the Kruskal-Wallis test and significant results were analyzed by the Tukey test. The similarity indexes

among steroid treatments and among populations were calculated as suggested by Sneath and Sokal (1973).

Wright (1963) reported 10 bands of esterase activity for *D. melanogaster*, which he numbered from 1-10, while Beckman and Johnson (1964) only found six esterase isoenzymes, which they identified with the letters from A to F. We used Beckman and Johnson's isoesterase classification but, to avoid confusion between F of fast population and F esterase isoenzyme, we named this esterase as Fs.

## RESULTS

All comparisons among isoenzyme patterns showed a significant effect of the hormonal treatments in the esterase pattern activation in the four instars, except for the third instar larvae of the F population (Tables I and II).

### Third instar larval

In the F population, the B, D, E and Fs isoenzymes were active in the untreated sample. We did not find significant changes in isoenzyme expression in the presence of non-endogenous steroids. In the C population, B, E and Fs isoenzymes were active in the untreated sample. With the exception of the untreated and ecdysone treated samples, all comparisons between treatments showed significant differences in the activation pattern in at least one band. Vitamin D3 and *S. malacoxylon* induced greater expression of the B isoenzyme than the other treatments, and activated esterase C. The S population presented two isoesterases in the untreated sample (E and Fs). With the exception of vitamin D3 and the untreated sample the other treatment comparisons showed significant changes in the E band activation with decreased activity in ecdysone treated sample and increased activity in *S. malacoxylon* treated sample (Figure 1A). In the vitamin D3 and *S. malacoxylon* treatments EST-B activity was detected.

### Stage 1 Pupae

Three isoenzymes (A, E and Fs) were detected in the F population at this stage. Vitamin D3 increased the expression of isoenzyme A when compared with untreated samples. The other treatments did not activate this isoenzyme. B, D, E and Fs esterases were detected in the untreated samples of the C population. We found wide variations in the activation pattern of these bands when samples treated with steroids are

analyzed in this stage. Vitamin D3 provoked greater changes in isoenzyme expression. In the S population the C, D, E and Fs isoenzymes were detected in the untreated sample; highly active B isoenzyme was detected with vitamin D3 treatments. The C and S populations were more responsive to hormonal treatment, in this ontogenetic stage, especially when treated with vitamin D3.

### Stage 2 Pupae

The F population in this stage showed weakly active esterase isoenzymes. The vitamin D3 treatment induced greater activity of isoenzymes A, E and Fs. Esterase A was observed only in the untreated and vitamin D3 samples, while with ecdysone and *S. malacoxylon* treatments it was inhibited. In the C population, isoenzymes B, E and Fs were active in all the samples but vitamin D3 treatment decreased the activity pattern of esterase B. The other treatments did not significantly change isoenzyme expression. The S population presented B, E and Fs isoenzyme activity though only isoenzyme B was affected by steroids. The vitamin D3 and *S. malacoxylon* treatments increased the activity of this isoenzyme (Figure 2a).

### Adult stage

At this stage a dramatic change in the esterase pattern of the F and S populations was observed. In the F population, only band E was active in the untreated samples. The ecdysone and *S. malacoxylon* treatments were able to repress the expression of this band while vitamin D3 increased their activity and induced the C isoenzyme. Surprisingly, the pattern found in the S population was just the opposite because vitamin D3 treatment inhibited the esterases, whereas the other treatments increased their activity (Table II). The effect of steroids on the C population was less conspicuous because esterase D was more active in the untreated sample than in the other treatments.

The similarity index effectively demonstrated the presence or absence of esterase isoenzymes among treated or untreated populations (Table III).

In the F population the greatest similarity was found between the ecdysone and *S. malacoxylon* treatments and the lowest indexes were between vitamin D3 and ecdysone and between vitamin D3 and *S. malacoxylon* (Table III). In the C population the highest similarity value was between the untreated sample and that treated with ecdysone. Among the remaining values the comparison with vitamin D3 was generally the lowest. In the S population the highest similarity

**Table I** - Comparison of chronological electrophoretic esterase activation patterns in four ontogenetic stages of *Drosophila melanogaster* populations selected for fast and slow developmental time and treated with steroids.

Pop	Comparison between treatments	Ontogenetic stages																							
		3rd INSTAR LARVAE isoenzymes						PUPA I isoenzymes						PUPA II isoenzymes						ADULT isoenzymes					
		B	C	D	E	Fs		A	B	C	D	E	Fs	A	B	C	D	E	Fs	A	B	C	D	E	Fs
F	U x Ec	ns	--	ns	ns	ns	7.7**	--	--	--	ns	ns	14.4***	--	--	--	7.2**	ns	ns	14.4***	--	ns	ns	12.9***	--
	U x V	ns	--	ns	ns	ns	12.2***	--	--	--	ns	ns	ns	--	--	--	14.6***	13.3***	ns	ns	--	17.9***	--	7.4**	--
	U x Sm	ns	--	ns	ns	ns	8.6**	--	--	--	ns	ns	14.3***	--	--	--	ns	ns	ns	14.3***	--	ns	ns	12.9***	--
	Ec x V	ns	--	ns	ns	ns	19.9***	--	--	--	ns	ns	16.6***	--	--	--	7.3**	11.9***	ns	16.6***	--	17.1***	--	20.3***	--
	Ec x Sm	ns	--	ns	ns	ns	ns	--	--	--	ns	ns	ns	--	--	--	9.8**	ns	ns	ns	--	ns	ns	ns	--
C	V x Sm	ns	--	ns	ns	ns	20.7***	--	--	--	ns	ns	16.6***	--	--	--	17.1***	9.3**	ns	16.6***	--	14.8***	--	20.3***	--
	U x Ec	5.4**	13.7***	--	ns	ns	--	6.5*	--	ns	6.3*	ns	--	--	--	ns	ns	ns	--	--	ns	ns	6.9***	ns	
	U x V	14.4***	5.1**	--	3.8**	18.5***	--	9.8**	--	15.3***	20.2***	12.9**	--	--	--	16.7***	ns	ns	--	--	20.1***	20.1***	11.3***	ns	
	U x Sm	4.8**	14.6***	--	ns	12.4***	--	8.8**	--	7.2**	ns	ns	--	--	--	ns	ns	ns	--	--	11.8***	11.8***	ns	ns	
	Ec x V	10.0**	6.6**	--	4.6*	15.2***	--	ns	--	14.8***	13.6**	19.2***	--	--	--	15.6***	ns	ns	--	--	13.2***	13.2***	8.8***	ns	
S	Ec x Sm	5.1**	8.6**	--	10.4***	ns	--	ns	--	8.1**	14.3**	9.9**	--	--	--	ns	ns	ns	--	--	4.8**	4.8**	ns	ns	
	V x Sm	ns	--	--	10.4***	ns	--	ns	--	11.2***	13.0***	9.9**	--	--	--	ns	ns	ns	--	--	8.3**	8.3**	11.3***	ns	
	U x Ec	17.9***	--	--	ns	ns	--	14.8***	10.7***	16.8***	ns	ns	ns	ns	ns	6.1**	ns	ns	ns	ns	3.8*	3.8*	4.2*	ns	
	U x V	12.9***	--	--	10.7***	ns	--	ns	ns	ns	ns	ns	ns	ns	ns	11.8***	ns	ns	ns	ns	13.4***	13.4***	19.3***	18.3***	
	U x Sm	17.9***	--	--	9.6**	ns	--	4.4*	ns	3.7*	ns	ns	ns	ns	ns	6.1**	ns	ns	ns	ns	6.7**	6.7**	4.2*	ns	
Ec x V	ns	--	--	21.1***	ns	--	ns	9.3**	10.6***	ns	ns	ns	ns	ns	5.7**	ns	ns	ns	ns	9.6**	9.6**	15.2***	18.3***		
	ns	--	--	21.1***	ns	--	15.8***	8.8**	14.4***	ns	ns	ns	ns	ns	5.7**	ns	ns	ns	ns	ns	ns	ns	ns		
	ns	--	--	21.1***	ns	--	ns	8.8**	14.4***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	6.7**	6.7**	15.2***	18.3***		

F, C, S = Fast, control and slow populations. Treatments: U = untreated; Ec = ecdysone; V = vitamin D3; Sm = *Solanum malacoxylon*-glycoside. A, B, C, D, E, Fs = isoenzymes. (-) Isoenzyme band not detected; ns = not significant with Kruskal-Wallis and Tukey test, \* = P < 0.05; \*\* = P < 0.01; \*\*\* = P < 0.001.

Table II - Isoenzymatic activity in four ontogenetic stages of *Drosophila melanogaster* populations selected for fast and slow developmental time and treated or not with steroids.

Third instar larvae																														
Pop.	Untreated						Ecdysone						Vitamin D3						<i>S. malacoxydon</i>											
	A	B	C	D	E	Fs	A	B	C	D	E	Fs	A	B	C	D	E	Fs	A	B	C	D	E	Fs						
F	--	○	--	○	○	○	--	○	--	○	○	○	--	○	--	○	○	○	--	○	--	○	○	○	--	○	--	○	○	○
C	--	○	--	--	○	○	--	○	--	--	○	○	--	↑	↑	--	○	○	--	↑	↑	--	○	○	--	↑	↑	--	○	○
S	--	--	--	--	○	--	--	--	--	--	○	--	--	↑	--	--	○	--	--	↑	--	--	○	--	--	↑	--	--	↑	--

Pupae 1																														
Pop.	Untreated						Ecdysone						Vitamin D3						<i>S. malacoxydon</i>											
	A	B	C	D	E	Fs	A	B	C	D	E	Fs	A	B	C	D	E	Fs	A	B	C	D	E	Fs						
F	○	--	--	--	○	○	↓	--	--	--	○	○	↑	--	--	--	○	○	↓	--	--	--	○	○	↓	--	--	--	○	○
C	--	○	--	○	○	○	--	○	--	○	○	--	--	○	--	↓	↓	--	--	--	○	--	○	○	--	○	--	○	○	--
S	--	○	○	○	○	○	--	○	○	↑	○	○	--	↑	○	↑	○	○	--	--	○	○	○	○	--	○	○	○	○	○

Pupae 2																															
Pop.	Untreated						Ecdysone						Vitamin D3						<i>S. malacoxydon</i>												
	A	B	C	D	E	Fs	A	B	C	D	E	Fs	A	B	C	D	E	Fs	A	B	C	D	E	Fs							
F	○	--	--	--	○	○	↓	--	--	--	○	○	↑	--	--	--	○	○	↓	--	--	--	○	○	↓	--	--	--	○	○	
C	--	○	--	--	○	○	--	○	--	--	○	○	--	↓	--	--	○	○	--	○	--	--	○	○	--	○	--	--	○	○	
S	○	○	--	--	○	○	○	○	--	--	○	○	○	○	↑	--	--	○	○	○	↑	--	--	○	○	○	↑	--	--	○	○

Adult stage																														
Pop.	Untreated						Ecdysone						Vitamin D3						<i>S. malacoxydon</i>											
	A	B	C	D	E	Fs	A	B	C	D	E	Fs	A	B	C	D	E	Fs	A	B	C	D	E	Fs						
F	--	--	--	--	○	--	--	--	--	--	↓	--	--	--	↑	--	↑	--	--	--	--	--	↓	--	--	--	--	--	↓	--
C	--	--	--	○	○	○	--	--	--	↓	○	○	--	--	↓	↓	↓	○	--	--	--	--	○	○	--	--	--	--	○	○
S	--	--	--	○	○	○	--	--	--	↓	○	○	--	--	--	↓	↓	↓	--	--	--	--	↓	○	--	--	--	--	○	○

Populations: F = fast; C = control; S = slow. (○) = Normal enzymatic activity; (--) = no enzymatic activity. (↑) = increased activity; (↑) = high activity; (↓) = decreased enzymatic activity; (↓) = weak activity.

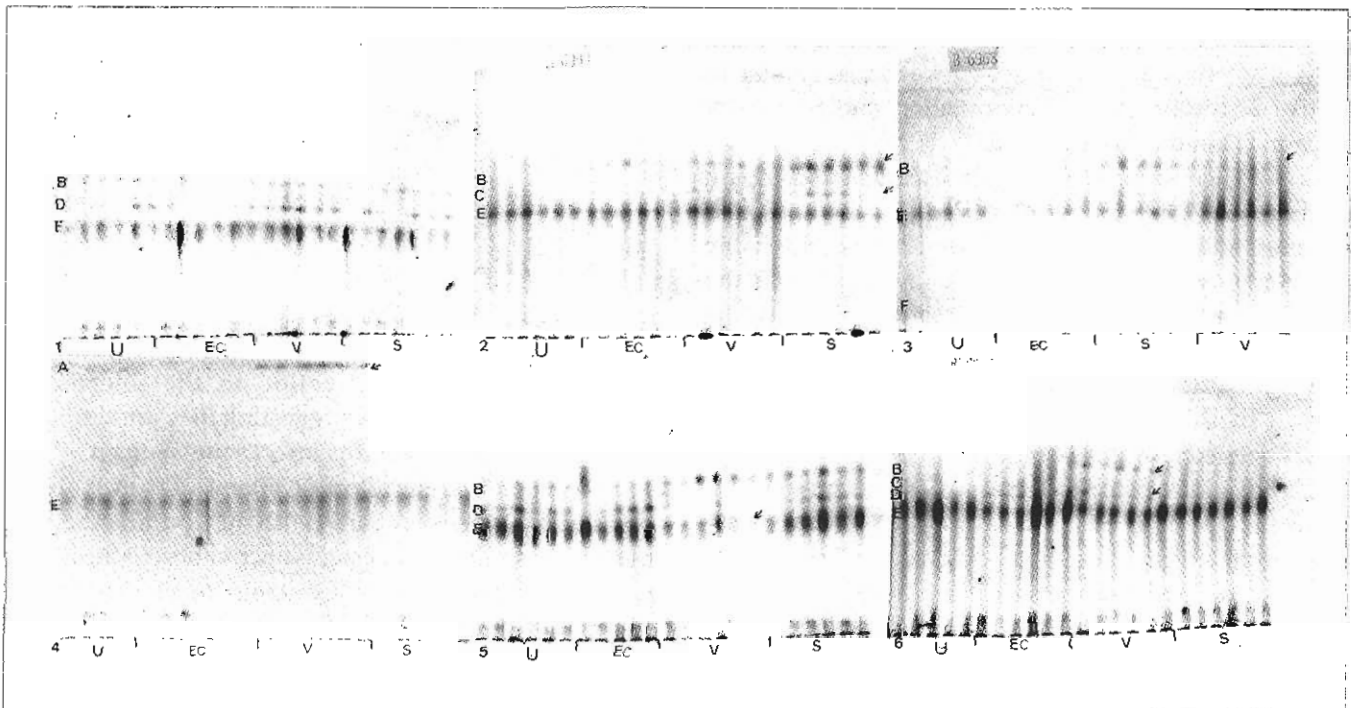


Figure 1 - Esterase pattern at the end of the third larval instar (above) and Pupa 1 (with adult eye developed) (below) of *Drosophila melanogaster* populations selected for fast and slow developmental rate and treated with steroids: 1 and 4 = fast population; 2 and 5 = control population; 3 and 6 = slow population. Treatments: U = untreated; Ec = ecdysone; V = vitamin D3, S = *Solanum malacoxydon*. The arrows indicate differences among activation pattern of the treatments.

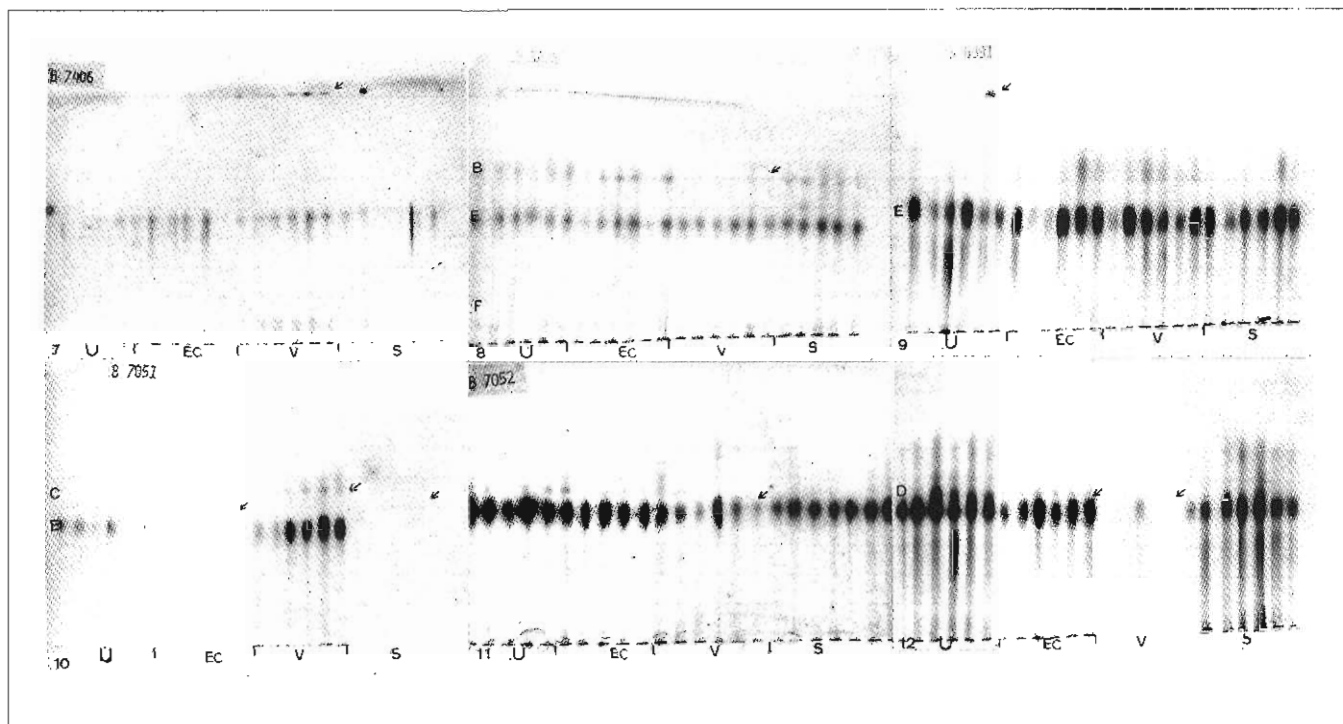


Figure 2 - Esterase pattern at the end of Pupa 2 (pharate pupae) (above) and just eclosed adult (below) of *Drosophila melanogaster* populations selected for fast and slow developmental rate and treated with steroids: 7 and 10 = fast population; 8 and 11 = control population; 9 and 11 = slow population. Treatments: U = untreated; Ec = ecdysone; V = vitamin D3; S = *Solanum malacoxylon*. The arrows indicate differences among activation pattern of the treatments.

occurred between the untreated samples and samples treated with ecdysone, vitamin D3 and *S. malacoxylon*.

In summary, we may conclude that vitamin D3 promoted greatest differentiation in F and C populations. The effect of *S. malacoxylon* was most similar to that of ecdysone in the F population and to that of vitamin D3 in the S population.

When we used the similarity index to compare populations (Table IV) we observed that the untreated samples of F and C populations showed the same number of activated esterase isoenzymes during the developmental stages studied. *S. malacoxylon* was the most effective treatment for the differentiation of these two populations.

In the comparison of the number of active bands between samples of the F and S populations the similarity index of 0.64 decreased in the presence of vitamin D3. The other treatments increased the similarity between these two populations (Table IV).

Untreated samples of the C and S populations showed the same similarity index. The vitamin D3 treatment effectively increased the similarity.

The S population similarity differed from the remaining ones. Nevertheless, while the C population showed indexes more similar to those of the F population with ecdysone and vitamin D3, these two treatments presented inverse results in the comparisons

between F and S, and S and C populations. On the other hand, while the action of *S. malacoxylon* glycoside was similar to that of ecdysone in these two comparisons (S x F and S x C populations), this was not the case in the comparison between F and C populations, which showed that *S. malacoxylon* glycoside was most effective.

## DISCUSSION

Our results showed an effective differential action of vertebrate and plant steroids on the pattern of esterase isoenzyme activation in the selected *D. melanogaster* populations, suggesting they are similar to functional hormones in this insect's metabolism.

The different effect of vitamin D3 in F and C populations when compared with the S population, and the higher similarity in esterase activation pattern of *S. malacoxylon* and ecdysone treatments in the F population, while this similarity was higher with vitamin D3 in the S population, support the idea of hormonal differences between these two selected populations, as suggested by Oliveira and Cordeiro (1982b), Loreto *et al.* (1988) and Jung (1992).

1,25-dihydroxycholecalciferol-glycoside, present in *S. malacoxylon* extract, has an effect in

**Table III** - Simple concordance coefficient of Sneath and Sokal (1973) for esterase isoenzyme activation in four ontogenetic stages between treatments in *Drosophila melanogaster* populations selected for fast and slow developmental time.

Comparative treatments	Populations		
	Fast	Control	Slow
U	11	11	6
Ec	8	11	6
I	<b>0.73</b>	<b>1.00</b>	<b>1.00</b>
U	11	11	6
V	12	9	5
I	<b>0.92</b>	<b>0.82</b>	<b>0.83</b>
U	11	11	6
Sm	8	12	5
I	<b>0.73</b>	<b>0.92</b>	<b>0.83</b>
Ec	8	11	6
V	12	9	5
I	<b>0.67</b>	<b>0.82</b>	<b>0.83</b>
Ec	8	11	6
Sm	8	12	5
I	<b>1.00</b>	<b>0.92</b>	<b>0.83</b>
V	12	9	6
Sm	8	12	6
I	<b>0.67</b>	<b>0.92</b>	<b>1.00</b>

Treatments: U = untreated; Ec = ecdysone; V = vitamin D3; Sm = *Solanum malacoxylon*, I = similarity index.

**Table IV** - Simple concordance coefficient of Sneath and Sokal (1973) for esterase isoenzyme activation pattern in four ontogenetic stages of *Drosophila melanogaster* selected for fast and slow developmental time and treated with steroid hormones.

Populations compared	Treatments			
	Untreated	Ecdysone	Vitamin D3	<i>S. malacoxylon</i>
Fast	11	8	12	7
Control	11	11	9	11
I	<b>1.00</b>	<b>0.73</b>	<b>0.75</b>	<b>0.64</b>
Fast	11	8	12	7
Slow	7	7	7	6
I	<b>0.64</b>	<b>0.88</b>	<b>0.58</b>	<b>0.86</b>
Slow	11	13	9	10
Control	7	8	7	6
I	<b>0.64</b>	<b>0.61</b>	<b>0.78</b>	<b>0.60</b>

I = Similarity index.

vertebrates which is similar to atherosclerosis produced by the steroid hormone 1,25-dihydroxyvitamin D3 (Wasserman *et al.*, 1976). In our work we observed that while *S. malacoxylon* treatment was most effective in increasing the differences in esterase isoenzyme expression patterns between F and C populations, vitamin D3 was most effective when we compared the F and S populations. The comparison between S and C populations showed that vitamin D3 promoted the highest similarity.

These results are surprising, considering the specificity of steroids, their receptors, and the expected responses. However, recent findings have demonstrated greater complexity. Ecdysone receptors (*EcR*) in cultured cells require another nuclear receptor named ultraspiracle (*usp*). The hormone response is mediated, at least in part, by a functional *EcR*, consisting of a binary complex of *EcR* and *usp*. *EcR* may function as a homodimer in some response elements or as a heterodimer with a different nuclear receptor partner. Different classes of *EcR* heterodimer may lead to functionally distinct *EcR* actions, some of them being dependent on *usp* and others not (Yao *et al.*, 1992). Nuclear receptor heterodimer formation is a conserved mechanism found in both vertebrates and invertebrates, suggesting that *usp* may be an essential component of the ecdysone response. Yu *et al.* (1991) demonstrated that *usp* forms heterodimers with mammalian nuclear retinoic acid receptors, thyroid hormone receptors, peroxisome proliferator-activated receptor and vitamin D3 receptors as well. Laudet *et al.* (1992) constructed and compared the phylogenetic tree derived from two different domains of 30 nuclear receptor genes. The tree built from the DNA binding C domain clearly shows a common progeny of all nuclear receptors. These investigators showed that the vitamin D3 and ecdysone receptors seem to have DNA binding and hormone binding domains belonging to the same class and to have diverged at a very early stage of evolution. Thus, ecdysone receptors may perhaps bind vitamin D3, affecting metabolic events in *Drosophila*, as shown by our results.

Jung (1992) found that biological properties of these same *D. melanogaster* selected populations such as egg-larva, larva-pupa and pupa-adult developmental stage viability, egg-adult developmental rate, frequency of normal anterior spiracle eversion and normal pupal development were affected by hormonal treatments, suggesting a functional role of vitamin D3 in *D. melanogaster* development. On the other hand, *S. malacoxylon*, which is harmful to vertebrates, appeared to be beneficial to *Drosophila* because it maintained or increased pre-imaginal viability and developmental rate, and effectively decreased the frequency of

alterations in anterior spiracle eversion and pupal development.

Simon and Koolman (1989) reported pharmacological aspects of ecdysteroids in vertebrates and demonstrated ecdysteroid action, such as protein stimulation synthesis in rat liver (Burdette, 1964), increased growth of infant mice by long-term feeding (Hikino and Takemoto, 1972), decreased cAMP level in mouse plasma and tissue (Catalan *et al.*, 1979a,b), induction of acetylcholinesterase (Catalan *et al.*, 1984), etc. These results taken together with recent studies on function and structure steroid receptors support the theory that invertebrate hormones have various effects on vertebrate metabolic events, a concept of "hormonal heterophyly" originally proposed by Burdette (1974). This suggestion is supported by the action of ecdysone on mammalian cells at the molecular level (Yao *et al.*, 1992).

Based on experimental results obtained by other authors, Koch *et al.* (1979) demonstrated that all cell membranes are able to develop new receptor molecules with some kind of stimulation. Taking into account this suggestion, Buckmann (1987) proposed that hormone and receptor do not have the same phylogenetic age. Hormones and their receptors are strictly interdependent and this relationship led Umesomo and Evans (1989) to propose a simple pathway for the coevolution of receptor DNA binding domains and hormone-responsive gene networks. A single Gly to Glu change in the first zinc finger produces a receptor that recognizes both glucocorticoid and estrogen response elements. Further replacement of five amino acids in the stem of the second zinc finger transform the specificity to that of the thyroid hormone receptor. The high conservation of amino acids even among usually divergent receptors leads to suspect the presence of cryptic functions that have yet to be revealed. On the other hand, the experimental observations summarized in a review by Csaba and Nemeth (1980) suggest that influences acting on the receptor during the critical period of its maturation play a decisive role in its development and responsiveness, a phenomenon known as "hormonal imprinting". Molecules capable of binding to the receptor despite slight structural differences from the hormone may account either for deformation or amplification of the receptor, depending on the degree of difference and/or on the dose. The elucidation of the hormone-receptor evolution is directly associated with investigations on several organisms, considering both endogenous and non-endogenous hormones. Lenard (1992) assembled works about the action of vertebrate hormones on microbial cells. He found research about the action of

molecules similar to vertebrate steroid hormones being carried out on fungi, bacterial cells and yeast, suggesting that the evolutionary pathway of the vertebrate endocrine systems may be more ancient than previously thought. Some other investigations about the relationship of vertebrate hormone-receptors with more complex living systems, such as higher plants, are being carried out. Schena *et al.* (1991), working with the glucocorticoid receptor of *Arabidopsis thaliana*, suggested that the glucocorticoid receptor has a conserved mechanism in plant and animals, indicating that higher plants express a set of highly conserved cellular components.

The relationship between ecdysone and juvenile hormone during ontogenetic development, possible dimer formation between *Drosophila usp* and mammalian vitamin D3 receptors, the hormonal imbalance between the selected populations studied in this work, the possible role of esterases in juvenile hormone degradation (Berger and Canter, 1973) and the different patterns of esterase activation found by us after hormonal treatment suggest an effective action of the non-endogenous steroids vitamin D3 and vitamin D3-glycoside on *Drosophila* metabolism.

Morgan (1926) stated that to understand development one must understand the molecular basis of differential gene expression. Although animals develop in very diverse ways, the discovery of related molecules in a wide range of species and, at the same time, molecules of several animal and plant origin with some functional role in the development of different organisms suggests that molecular mechanisms underlying developmental and physiological homeostasis may be much more universal and conserved than was previously suspected, as also suggested by the present results.

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## RESUMO

Populações de *Drosophila melanogaster* selecionadas durante 27 anos para velocidade de desenvolvimento rápido e lento foram tratadas com hormônios esteróides (ecdisona,

vitamina D3 e vitamina D3 glicosídica presente na planta *Solanum malacoxylon*) com o objetivo de verificar o seu efeito no padrão de esterase em quatro estágios ontogenéticos (fim do terceiro estágio larval, pupa com olhos diferenciados, pupa farata e adulto recém-eclodido). Observou-se que a vitamina D3 promoveu as maiores diferenças no padrão de atividade esterásica nas populações rápida e controle. O efeito do *S. malacoxylon* foi mais semelhante ao da ecdisona na população rápida, enquanto na população lenta este efeito foi mais semelhante à vitamina D3. Quando o índice de similaridade foi usado para comparar as populações verificou-se que, enquanto entre as populações rápida e controle o tratamento com *S. malacoxylon* foi mais efetivo no padrão de ativação de esterase, entre as populações rápida e lenta o tratamento com vitamina D3 foi mais eficiente. Na comparação entre as populações lenta e controle, com exceção do tratamento com vitamina D3, os demais tratamentos bem como a amostra não tratada mostraram níveis semelhantes de ativação. Sugere-se portanto ação hormonal efetiva de esteróides de vertebrados e plantas no metabolismo de *D. melanogaster*.

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