

A SECOND CHROMOSOME MAJOR GENE FOR DEVELOPMENTAL RATE IN *Drosophila melanogaster*

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ABSTRACT

Drosophila melanogaster Oregon R lab stock was subjected to mass selection for many generations for slow and fast developmental rate. Crossing of homozygous "slow" females with heterozygous males (fast/slow) showed a bimodal curve for developmental rate, indicating a major gene effect (Oliveira and Cordeiro, *Rev. Bras. Biol.* 41: 635-644, 1981). This paper reports the experiments with discrete eclosion classes that located the gene for slow developmental rate on the second chromosome by use of an enzymatic as well as body markers. With a three point test the position was determined at 63.8. This mapping is requisite for a future molecular procedure to isolate, clone and sequence this "slow gene", as well as to study its action.

INTRODUCTION

Developmental rate is an important fitness component, notorious for its small phenotypic variance and resistance to artificial selection. Genetic control of developmental rate by polygenes is supported by many papers (Robertson, 1963, 1964; Marinkovic and Ayala, 1986a,b). Our knowledge of this character increased with the discovery of regulatory control of developmental rate (Della Crosse *et al.*, 1975; Jayakar *et al.*, 1977).

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The influence of enzyme polymorphism on developmental rate was also suggested by other authors (Marinkovic and Ayala, 1975a,b; Cavener, 1983; Marinkovic *et al.*, 1987). The finding by Oliveira and Cordeiro (1981) of a major gene control of developmental rate was quite suggestive of a regulatory locus that expresses itself clearly in "slow cytoplasm", producing a bimodal curve (homozygote slow females x heterozygote fast/slow males).

Regulatory genes are tentatively defined by Hedrick and McDonald (1980) as those loci whose primary role is to control the timing or degree of expression of the products of other genes. Promoters are such DNA sequences and are now being widely used to enhance gene expression in transgenic organisms.

The chromosomal location and genetic mapping of the slow developmental rate allele in *D. melanogaster* is presented in this paper and its role as a structural or regulatory gene for a quantitative trait is discussed.

MATERIAL AND METHODS

We used *D. melanogaster* Oregon-R populations that had been selected during 554 and 395 generations for fast and slow developmental rate, respectively. These selected populations were named (PP₁) = fast population and (tt₁) = slow population.

Before selection, the egg-adult cycle at 25°C was 10 days. At this temperature, after selection, the fast population adults started eclosion on the 7th day after oviposition, whereas the slow population flies began eclosion only on the 12th day, when both precocious and unselected control flies all already eclosed. There was therefore, no possibility of phenotypic overlap of the genotypes which control developmental rate or, consequently, misclassification.

To maintain the fast development (PP₁) cultures, we bred the first flies that eclosed, especially those of the 8th and 9th days, let the flies age during two days and then made three culture bottles of 1/4 l capacity with nearly 100 individuals each that were transferred every three hours to new bottles. For the maintenance of the slow population (tt₁), we bred only the last flies that eclosed, on the 13th day or afterwards. The transfer of these cultures were made every six hours which maintains about the same level of larval competition of the more fertile fast population.

To ascertain the number of genes involved in the control of developmental rate, we made a new selection starting with the F₁ of crosses between fast (PP₁) and slow (tt₁) flies, selected during 227 and 147 generations respectively. These flies were crossed in both directions: female fast x male slow and female slow x male fast. The offspring of these crosses were then reselected for fast and slow development. With the new selection

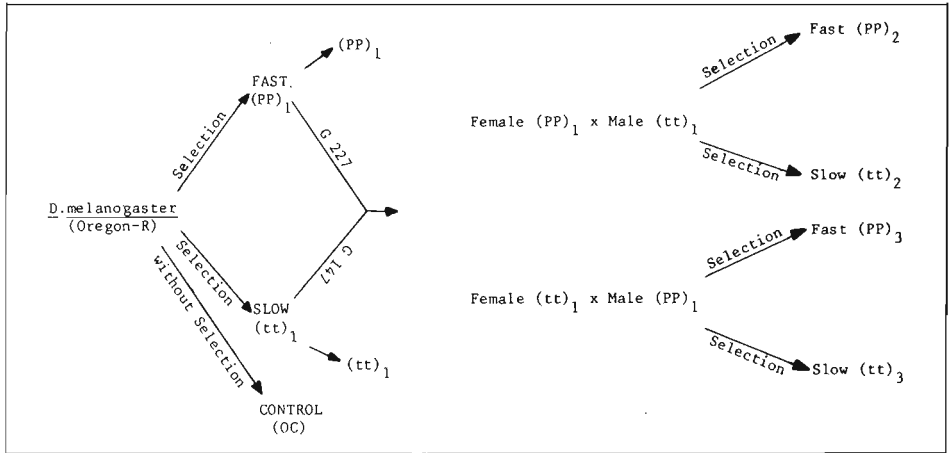


Figure 1 - Origin of the populations (PP)₁, (PP)₂, (tt)₁ and (tt)₃ of *D. melanogaster* used in this study.

we obtained four populations that were designated: (PP)₂, (PP)₃, (tt)₂ and (tt)₃, as summarized in Figure 1. In this work we used only the (PP)₁, (tt)₁, (PP)₂ and (tt)₃ populations.

Chromosomal location using enzymatic markers

The *D. melanogaster* fast population (PP)₁ is monomorphic for the IDH-NADP slow allele (S), while the population selected for slow developmental rate is monomorphic for the fast allele (F) of that enzyme (Loreto and Oliveira, 1988). Isozyme patterns were obtained by electrophoresis on 6% polyacrilamide gel, using the continuous buffer system of Huettel and Bush (1972). A field of 10 V/cm was applied and the running distance was 9 cm. The gels were then developed in a mixture of 150 mg isocitric acid; 20 mg NBT, 20 mg NADP, 20 mg MgCl₂ and 5 mg PMS buffered in 0.1 M Tris HCl, pH 8.65. Males and females of the two selected populations ((PP)₁ and (tt)₁) were crossed in both directions and F₁ males and females were backcrossed with the original selected flies. Sample offspring of the eight backcrosses between the two selected populations, maintained isolated until the last eclosion day, were analysed for the IDH-NADP allelic pattern. We mainly observed the first and the last flies that eclosed with the expectation that, if the developmental rate gene is linked to the IDH structural locus, which is located on the 3rd chromosome (3.27.1), in the backcrosses with the slow population, the first flies to eclose would be heterozygotes and would show the heterozygote pattern for IDH-NADP (F/S), while the last ones would be the slow homozygotes and would display the fast allele only (F/F).

Chromosome location using phenotypic body markers

The Cy L/Pm stock (Cy = Curly, 6.1 2nd, wings curled upward; L = Lobe, 72.0 2nd, reduced eyes; Pm = Plum 104.5 2nd, eye color like bw or pr, mottled with darker spots that become more red with age) was used. Cy L/Pm males were crossed with slow females (tt)₃ and F₁ males were backcrossed with slow (tt)₃ females. Ten pairs of flies were distributed by vial and 72 culture bottles were obtained by daily transfers. F₂ phenotypes were observed and scored.

Genetic mapping

Sp J/Gla stock (Sp = Sternopleural, 22.0 2nd, higher number of sternopleural bristles; J = jammed, 41.0 2nd, wings often compressed into narrow strips, sometimes filled with fluid, allula larger and square tipped with clumped bristles and bare regions) was used for the three-point test. Marked males were crossed with slow reselected females (tt)₃ and the F₁ females backcrossed with slow males (tt)₃. To obtain the F₁, ten couples were put in each of 48 vials; for the F₂, 10 couples were put in each of 96 vials. To identify the F₂ the following procedures were carried out: the F₂ was developed at 28°C to allow the Sp phenotype to be identified. F₂ males and females discriminated for the two body markers were crossed with slow reselected flies (tt)₃; 5,000 individual crosses were made, each with a single couple per vial, to detect the tardiness of the descendents at 25°C.

RESULTS

Chromosomal location using enzymatic markers

Table I gives the results of the electrophoretic analysis of the isocitrate dehydrogenase NADP dependent (IDH-NADP) phenotypes made with samples of the eight backcross types possible between the two selected populations.

As can be seen, especially in the backcross where slow flies were used, there are fast homozygotes for IDH-NADP (F/F) even among fast flies, i.e., those eclosed on the eighth day after oviposition (crosses 1, 3, 5 and 7). Similarly, homozygous flies for the slow IDH-NADP alleles (S/S) and heterozygous for the fast and slow allele (F/S) of this enzyme extend eclosion until the fifteenth day after oviposition (crosses 2, 4 and 8), which demonstrates that the major gene responsible for the population tardiness is probably not linked to the structural gene that codes IDH-NADP, and thus is not located on the 3rd chromosome. As was shown by Oliveira and Cordeiro (1981), the genes for fast development are dominant over those for slow development, so it was expected that the heterozygous individuals would be fast.

Table 1 - Relative phenotype frequencies of fast and slow alleles of IDH-NADP in backcrosses of *D. melanogaster* populations selected for fast developmental rate (PP)₁ and slow developmental rate (tt)₁. The data refer to the % calculated for the total flies analyzed in each cross.

Cross	IDH-NADP Phenotype	Ecdysis day										Total	
		7	8	9	10	11	12	13	14	15	16		
		%	%	%	%	%	%	%	%	%	%	%	%
1 ♀ Pt x ♂ (tt) ₁	F		11.21			9.35	28.04						
	FS		26.17			14.02	11.21						
	S		-			-	-						
	Total		37.38			23.37	39.25						107
2 ♀ Pt x ♂ (PP) ₁	F		-		4.54	-	-		11.40	-	-	-	-
	FS		21.59		4.54	-	-		11.40	-	25.00	-	25.00
	S		19.32		-	-	-		-	1.14	17.04	-	17.04
	Total		40.91		4.54	-	-		11.40	1.14	42.04	-	42.04
3 ♀ Pt x ♂ (tt) ₁	F		7.27	3.64	1.82	3.64			1.82				1.82
	FS		38.18	1.82	3.64	3.64			18.18	3.64			3.64
	S		-	-	-	-			-	-			-
	Total		45.45	5.46	5.46	7.28			20.00	3.64			1.82
4 ♀ Pt x ♂ (PP) ₁	F		-		-	-			-				-
	FS		21.54		4.61				6.15				15.38
	S		12.30		1.54				1.54				23.10
	Total		33.84		6.15				7.69				38.48
5 ♀ Pt x ♀ (tt) ₁	F		30.00	16.67							6.67		
	FS		30.00	13.33							3.33		
	S		-	-					-		-		-
	Total		60.00	30.00					10.00				30

Continued

Table I - Continued.

Cross	IDH-NADP Phenotype	Ecdysis day											Total	
		7	8	9	10	11	12	13	14	15	16			
		%	%	%	%	%	%	%	%	%	%	%	%	%
6 ♂ Pt x ♀ (PP) ₁	F	-	-	-	-	-	-	-	-	-	-	-	-	-
	FS	29.41	29.41	20.20	21.57	5.88	1.96	-	-	-	-	-	-	-
	S	20.41	20.41	23.10	11.80	-	-	-	-	-	-	-	-	-
	Total	58.82	58.82	43.30	33.37	5.88	1.96	-	-	-	-	-	-	-
7 ♂ tP x ♀ (tt) ₁	F	1.00	1.00	20.20	14.40	-	3.80	12.50	2.00	-	-	-	-	-
	FS	4.80	4.80	23.10	9.60	-	3.80	4.80	-	-	-	-	-	-
	S	-	-	-	-	-	-	-	-	-	-	-	-	-
	Total	5.80	5.80	43.30	24.00	-	7.60	17.30	2.00	-	-	-	-	-
8 ♂ tP x ♀ (PP) ₁	F	-	-	-	-	-	-	-	-	-	-	-	-	-
	FS	0.97	23.30	-	1.94	0.97	-	-	7.77	-	-	-	-	14.56
	F	10.68	17.47	-	1.94	0.97	-	-	2.91	-	-	-	-	16.50
	Total	11.65	40.78	-	3.88	1.94	-	-	10.68	-	-	-	-	31.07

(PP)₁ = (*D. melanogaster* selected for fast developmental rate); (tt)₁ = (*D. melanogaster* selected for slow developmental rate; tP = F₁ (♀ (tt)₁ x ♂ (PP)₁); Pt = F₁ (♂ (tt)₁ x ♀ (PP)₁).

The intermediate blank squares were not analyzed.

F = fast IDH-NADP allele; S = slow IDH-NADP allele.

Figure 2 shows the electrophoretic pattern of heterozygotes and homozygotes for IDH-NADP of the backcross F₁ female (female (tt)₁ x male (PP)₁) x male (tt)₁, and it can be seen that both phenotypes occur in flies that eclose on the eighth as well as on the fifteenth day after oviposition.

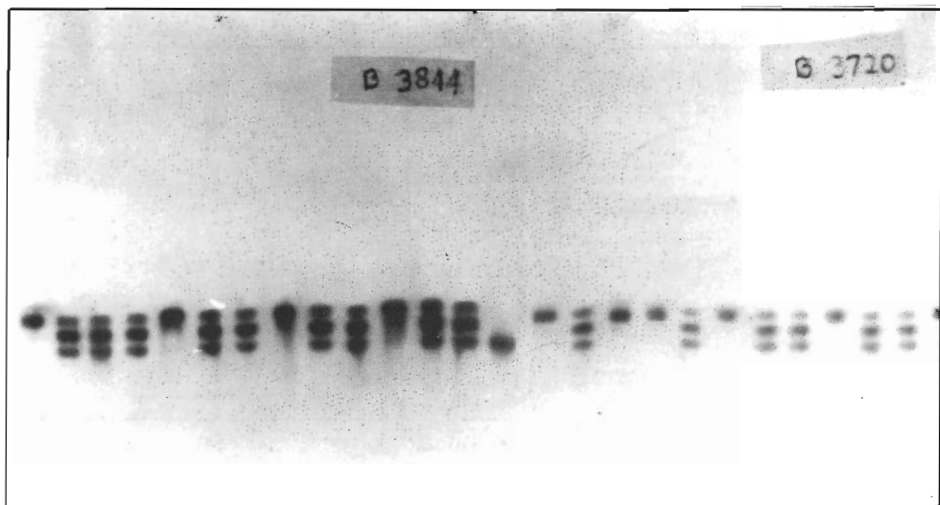


Figure 2 - Electrophoretic pattern of homozygotes and heterozygotes for IDH-NADP alleles showign that both phenotypes occur in flies that eclose on the eighth as well as on the fifteenth day after ovipostion. 1-3 eighth day male of the cross F₁ ♀ (♀ (tt)₁ x ♂ (PP)₁) x ♂ (tt)₁; 14 - Control (slow IDH-NADP allele of a monomorphic population OC; 15-26 - fifteenth day male of the same cross.

Chromosomal location using phenotypic body markers

Table II shows the results of the use of the 2nd chromosome phenotypic body marker.

As can be seen in Table II, the obtainment of two phenotypes only (mutant body phenotype and normal developmental rate, that is, eclosion between the 9th and 11th day after oviposition and wild body phenotype and slow developmental rate, that is, eclosion between the 12th and 14th day after oviposition) denotes that the main gene responsible for slow developmental rate is linked to the marker genes used, and hence must be located on the 2nd chromosome. The high χ^2 test value indicates that the lower viability of slow flies (Oliveira and Cordeiro, 1981) affects the observed numbers of the two phenotypic classes in F₂.

Table II - Observed numbers in backcross of F₁ 2nd chromosome marked male with double homozygous slow female.

Cross: ♂ F ₁ Cy/ + + / t x ♀ + / + t / t							
Eclosion days	9	10	11	12	13	14	
Phenotypes							
Cy	665	1459	103				
Slow				35	1232	180	
Total		2227	Cy		1447	slow	3674
χ^2					165.17 > $\chi^2_{.05 (1)} = 3,84$		

Cross: ♂ F ₁ LPm/ ++ + / t x ♀ ++ / ++ t / t							
Eclosion days	9	10	11	12	13	14	
Phenotypes							
L Pm	931	1236	65				
Slow				135	723	166	
Total		2232	LPm		1024	slow	3256
χ^2					447.44 > $\chi^2_{.05 (1)} = 3,84$		

t = slow developmental rate gene.

Recombination genetic mapping

The data in Table III were obtained from the body marker stock used. As the F₂ crosses were made individually, there was no competition and eclosion was nearly simultaneous. We considered as slow phenotype those flies which eclosed from the 12th on when the remaining flies were already all eclosed. This avoided phenotype overlap and parental and recombinant progeny misclassification.

Table III - Observed phenotypic frequencies in a three point test using F₁ females with a 2nd chromosome marker and the allele of the gene for slow developmental rate backcrossed with a slow homozygous male.

F ₂ Phenotype	F ₂ Genotype	Observed number				χ^2
		Male	Female	O	E	
Sternopleural, Jammed	$\frac{Sp J +}{++t}$	486	905			
				2297	2091.96	20.09
Slow	$\frac{++t}{++t}$	283	623			
Sternopleural, slow	$\frac{Sp + t}{++t}$	48	111			
				511	590.04	10.59
Jammed	$\frac{+ J +}{++t}$	106	246			
Sternopleural, Jammed, slow	$\frac{Sp J t}{++t}$	0*	0*			
				624	618.54	0.03
Wild	$\frac{+++}{++t}$	101	523			
Sternopleural	$\frac{Sp ++}{+++}$	5	30			
				43	174.46	99.06
Jammed, slow	$\frac{+ J t}{++t}$	1	7			
Total		1030	2445	3475	3475	129.77 > $\chi^2_{.05}(3) = 7.81$

* (Lethal ?).

The middle gene was jammed and the recombination rates between Sp and J were $0.147 + 0.012 = 0.159$ and between t (major gene for tardiness) and J $0.179 + 0.012 = 0.191$. Genetic distance between SP and J was 15.9 cM and between t and J 19.1 cM. The exact locations of SP and J were 22.0-2nd and 41.0-2nd, respectively, and as in this cross a lethal class (SP J t/++t) occurred, since the distance between SP and J, 19,

corresponded to 15.9 cM, that between t and J, 19.1, correspond to 22.82 cM, and the major slow gene was probably located at 63.82-2nd.

An intriguing observation (Table III) is the sex segregation distortion rate of males and females in F₂. The high χ^2 test indicates that the sex distortion rate and the lower viability of slow and body markers flies affect the observed number of distinct classes in the F₂.

DISCUSSION

The mapping of genes for quantitative traits is important for understanding their character and function (Thoday, 1961). Most quantitative traits are influenced by many genes (polygenic inheritance); however, the actual number of genes included and the magnitude of their individual effects is a subject of controversy (Falconer, 1981). The classical view is that hundreds of genes each with small, equal and additive effects are involved (Mather and Jinks, 1984), while it has also been proposed that quantitative traits are controlled by relatively few major genes, modified by minor genes (Thoday and Thompson, 1976, Shrimpton and Robertson, 1988).

Oliveira and Cordeiro (1981), and the present work, studying *D. melanogaster* populations selected for fast and slow developmental rate, found that there are probably two major genes controlling this parameter, and a recessive allele is responsible for slow development.

Della Crosse *et al.* (1975) found that a regulator system for developmental rate is located on the 3rd chromosome but Jayakar *et al.* (1977), who crossed a wild *D. melanogaster* Canton stock with 2nd and 3rd marker chromosomes, observed the association of developmental rate not only with a 3rd chromosome marker but also with a 2nd chromosome marker. This was confirmed by our data which indicate a late regulator gene to be located on the 2nd chromosome.

Marinkovic *et al.* (1987) examined eight enzyme polymorphisms in groups of *D. melanogaster* flies with fast, intermediate and slow development and found no significant differences in average heterozygosity between them. Nevertheless, they obtained evidence of genetic differentiation among developmental classes. The genotypic frequencies were significantly heterogeneous for each of the three loci on the second chromosome. None of the five third-chromosome loci showed significant heterogeneity by a simple χ^2 heterogeneity test.

Enzyme polymorphisms had been earlier implicated in the developmental rate of *D. melanogaster* (Cavener, 1983), as well as in *D. pseudoobscura* (Marinkovic and Ayala, 1975a,b). It may be that these loci themselves affect the developmental rate of the flies, but it is of course possible that other loci, closely linked with those examined, may actually be the ones directly involved in rate of development.

Given the small number of loci examined, we can not evaluate the relevance of the fact that all three 2nd chromosome loci, but none (if a χ^2 heterogeneity test is used) or only one (if the correlation test is used) of five 3rd chromosome loci give significant heterogeneity among developmental classes. This difference could be a sampling error, or it could be that rate of development depends on more loci located on the 2nd than on the 3rd chromosome, as our results have shown.

The fact that considerable frequencies of both fast and slow alleles can be found in natural populations of *D. melanogaster* is thus far a unique aspect of this kind of gene and favors continued investigation of questions concerning their difference.

The gene for slow developmental rate also appears to act since early development, and evidence supporting this is the increase of asymmetry when individuals with certain phenotypic body markers are crossed with (tt)₃ individuals. According to Andrade *et al.* (1979), when flies from stocks of *D. melanogaster* were crossed with slow flies (tt)₃, an increase in asymmetry occurred: CyL/Pm x (tt)₃ in F₁ produced 63% asymmetry for L phenotype expression; Pbx/Sb x (tt)₃ in F₁ produced 66.8% asymmetry for Pbx phenotypes, while the stock CyL/Pm itself produced 33.3% asymmetry for L and the Pbx stock itself produced 46% asymmetry for Pbx. The asymmetry produced in the F₁ of mutants x fast flies (PP)₂ was at the same levels found for mutant stock only: F₁ CyL/Pm x (PP)₂ produced 45.6% asymmetry for L and Pbx/SB x (PP)₂ produced 42.2% asymmetry for Pbx. Only the (tt)₃ population, when crossed with the mutants studied, greatly increased developmental disturbances, which could explain the lower viability of this population, referred to by Oliveira and Cordeiro (1981), and also observed in this experiment, where slow flies always eclosed in lower numbers. Low viability is probably responsible for the high χ^2 test values obtained for the slow gene location and for the different numbers observed in parental and recombinational classes, where flies carrying the slow gene occur in lower frequency.

The observed effect of this slow gene on developmental rate, viability, sex segregation distortion rate and asymmetric response of some phenotypic markers suggest that it is more probably a regulatory than a structural gene.

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RESUMO

Estoque Oregon-R de *D. melanogaster* foi submetido a seleção massal por muitas gerações espara velocidade de desenvolvimento rápido e lento. Cruzamentos de fêmeas homozigotas "lentas" com machos

heterozigotos (rápidos/lentos) mostraram uma curva bimodal para a velocidade de desenvolvimento indicando efeito de 2 genes maiores (Oliveira e Cordeiro, 1981). O presente trabalho descreve os experimentos que localizaram e mapearam o gene principal para velocidade de desenvolvimento tardio no II^o cromossoma a 63.8 usando técnicas genéticas clássicas e isocitrato desidrogenase dependente de NADP como marcador enzimático. Tal mapeamento possibilita procedimentos moleculares adicionais para isolar, clonar e sequenciar este gene assim como estudar o seu modo de ação.

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