With regard to the LAP, 3 areas of activity appeared (Figure 2), characterized as areas III, II, and I according to their increasing electrophoretic mobility. Area I consisted of 1 to 3 zones. This area was present in all developmental stages with a maximum of relative activity in the pupal stage. Area II consisted of one zone of stable mobility present in all stages, with a peak of activity in 1- and 4-day-old pupae. Finally, area III consisted of 4 zones having different times of appearance. Thus, zone III_1, with the faster mobility, was present from 4-day-old pupae and thereafter zones III_2 and III_3 appeared from 12-day-old larvae up to the adults, while III_4 was observed from 8-day-old larvae and thereafter. The maxima of relative LAP-activity were observed in 4- and 7-day-old pupae. In some of the samples examined of 1-day-old pupae a faint zone of an intermediate mobility of areas I and II was visible. Generally, the highest LAP-activity was present in the pupal stage, particularly in mid and late pupae. The electrophoretic pattern of LAP in *Plodia* is in agreement with the findings of SAKAI et al.[18] concerning the stage where the highest LAP-activity appeared. The above observation supports the suggestion of SAKAI et al.[18] about the participation of exopeptidases, controlled by LAP-D locus, in the massive histolysis of larval tissues in pupal stage.

The absence of some zones in esterase and LAP zymograms of eggs, early and intermediate larvae may not indicate total absence of these molecular forms, but could be due to the sensitivity of electrophoretic technique[24]. On the other hand, the constancy of the majority of the molecular forms of esterase and LAP activity during metamorphosis in *Plodia*, supports the suggestion of PANTELOURIS et al.[2] that the genes responsible for the synthesis of esterases must be 'on' before and during metamorphosis.

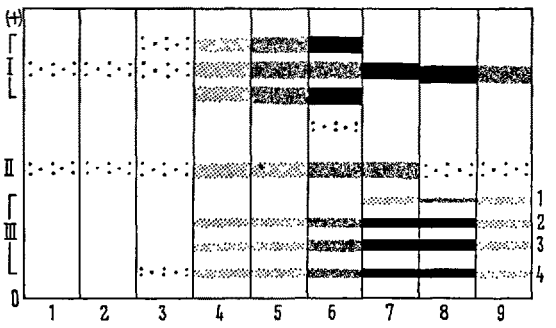

Fig. 2. Schematic picture showing the electrophoretic pattern of leucine aminopeptidase during ontogenesis of *Plodia interpunctella*. 1. Eggs; 2–5. Larvae (1-, 8-, 12- and 16-day-old respectively); 6–8. Pupae (1-, 4- and 7-day-old respectively); 9. Adults.

Zusammenfassung. Es wurden elektrophoretische Esterase- und Leucinaminopeptidase-Muster im Verlaufe der Entwicklung von *Plodia interpunctella* dargestellt.

H. A. PATARYAS[25], A. A. HARITOS and H. GELTI-DOUKA

Department of General Biology, University of Athens, Solonos 57, Athens (Greece), 2 September 1970.

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[25] Present address: Department of Medicine, University of Washington, Division of Medical Genetics, Seattle (Washington 98105, USA).

DNA and RNA Content in Diploid and Tetraploid Amphibians

The first cytological demonstration of polyploidy in vertebrates was reported by BEÇAK et al.[1] in the anuran *Odontophrynus americanus*. Several other examples of polyploidy were subsequently shown in other amphibians of the family Ceratophrydidae[2,3], Hylidae[4,5], and in fish[6,7].

The genus *Odontophrynus* includes several diploid species, as *O. cultripes*, *O. carvalhoi*, *O. occidentalis* and *O. americanus*, each with $2n = 22$ chromosomes[2,4,8]. The latter is confined to just a few localities. Another population of *O. americanus*, whose specimens are very similar to the diploid ones, was found to be tetraploid, exhibiting $4n = 44$ chromosomes[1,2]. The $4n$ populations have a wide geographical distribution, including several South American countries.

The existence of 2 populations phylogenetically very closely related, showing however different degrees of ploidy, constitutes an ideal system for a comparative study on gene expression. This peculiar situation gives rise to a few interesting questions. What happens to the mechanisms of regulation and transcription after the species duplicates all its genetical material? Is the production of RNA, proportional to the level of ploidy? Does the amount of protein of the tetraploid correspond to the DNA increase?

Starch gel electrophoresis of the albumin-like protein of the $4n$ *O. americanus* revealed polymorphism at this locus. 5 distinct phenotypes were found, showing that all 4 homologue genes are active in each animal but not necessarily synchronical[9].

RNA content. RNA measurements were made by spectrophotometric determinations according to the modified method of SCHMIDT and TANNHAUSER[10,11]. The animals were dissected and the kidneys immediately frozen. The tissue was homogenized in distilled water and the homogenate precipitated in $0.6N$ perchloric acid, centrifuged at $10{,}000 \times g$ for 10 min at 4 °C and washed in $0.2N$ perchloric acid. The precipitate was

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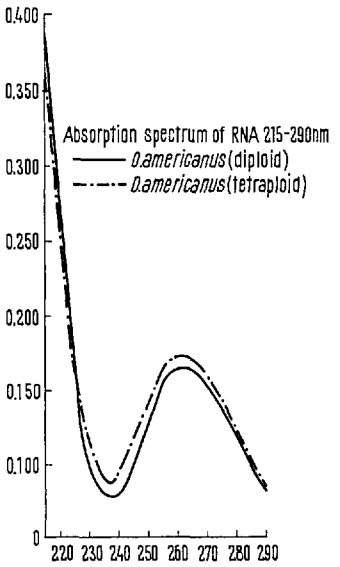
Table I. DNA and RNA content in the diploid *O. americanus*

Specimen	DNA content (γ/mg tissue)	RNA content (γ/mg tissue)	RNA / DNA	DO 260 / DO 280
1	0.48	5.47	11.21	1.35
2	0.61	5.66	9.25	1.39
3	0.59	5.40	9.10	1.34
4	0.55	6.04	10.99	1.34
5	0.53	4.99	9.38	1.35

Table II. DNA and RNA content in the tetraploid *O. americanus*

Specimen	DNA content (γ/mg tissue)	RNA content (γ/mg tissue)	RNA / DNA	DO 260 / DO 280
1	1.17	6.62	5.65	1.36
2	1.06	4.92	4.66	1.38
3	1.24	5.28	4.24	1.40
4	1.04	4.92	4.73	1.38
5	1.03	4.80	4.66	1.38
6	1.20	5.40	4.50	1.40
7	1.06	4.80	4.50	1.38
8	1.08	6.27	5.78	1.35
9	1.14	4.92	4.30	1.38
10	1.09	4.60	4.21	1.37
11	1.00	5.24	5.22	1.34
12	0.99	5.08	5.11	1.36
13	1.14	5.88	5.13	1.34
14	0.97	5.69	5.83	1.35



Comparative absorption spectrum of RNA at the wave-length of 215–290 nm, for the diploid and the tetraploid *O. americanus*.

hydrolyzed with 0.3 *N* KOH at 37 °C for 60 min. Proteins and DNA were precipitated by 1.2 *N* perchloric acid, centrifuged and the sediment washed in 0.2 *N* perchloric acid. The supernatant and the washing fluids were diluted to a final concentration of 0.1 *N* perchloric acid. The determinations were made in a Zeiss PMQ II spectrophotometer at a wave-length of 215–290 nm. The results obtained, measuring 14 tetraploid and 5 diploid specimens, are shown in Tables I and II and in the Figure.

The mean RNA content determined was 5.516 γ/mg, and 5.320 γ/mg for kidney tissue of the diploid and tetraploid, respectively. The mean differences are not significant ($t = 1.29$).

The relative protein contents in specimens of both types of populations were practically the same. The mean values (DO 260/DO 280) were 1.35, and 1.36 for diploids and tetraploids, respectively.

DNA content. DNA was determined by the method of CERIOTTI[12] based on the indol reaction with the desoxiribose of the precipitate, obtained by 1.2 *N* perchloric acid treatment of the KOH hydrolisate. The precipitate was resuspended in 0.3 *N* KOH and diluted to 0.1 *N* KOH[10]. 2 ml of this solution were treated with 1 ml of 0.04% indol and 1 ml of concentrated HCl, incubated for 10 min at 100 °C and extracted with purified chloroform[12]. Optical density was measured in a Zeiss PMQ II spectrophotometer.

The DNA contents are shown in Tables I and II. The mean contents were determined as 0.552 γ/mg and 1.086 γ/mg for kidney tissue of the diploid and tetraploid, respectively.

Therefore the ratio of DNA values of the $2n$ and $4n$ specimens is 1:2. A similar ratio was found by cytophotometric measurements of nuclear DNA content of Feulgen stained erythrocyte preparations[8].

The relative RNA and DNA content in the specimens of either population may be expressed by the proportion RNA/DNA. The values are 9.98 for the diploid and 4.82 for the tetraploid.

The results showed that the actual RNA amount in the tetraploid *O. americanus* is almost the same as in the diploid one. The RNA level remains constant independent of the degree of ploidy.

The peculiar gene expression in the tetraploid *O. americanus* may hypothetically be explained by an asynchrony of genomes or a random inactivation of homologue genes in each animal. Alternatively, it may be due to a higher concentration of repressors or a reduced transcription rate of the duplicate genome, as a buffer action in relation to the excess of genetic material[13,14].

Resumen. En el anuro tetraploide natural *Odontophrynus americanus*, el contenido de DNA es el doble que el determinado, bioquimicamente, en los especimens de la población diploide de *O. americanus*. Sin embargo el contenido de RNA de ejemplares de las dos poblaciones es aproximadamente igual. Aparentemente, mecanismos de regulación bloquean el esceso de material genético en el tetraploide.

W. BEÇAK and G. GOISSIS

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