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Base analysis of deoxyribonucleic acid of several insects and spider testis

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몇가지 昆蟲 및 蜘蛛의 精巢 DNA 鹽基에 關한 硏究

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摘 要

家蠶(蛹體), 벳뚜기 2種 및 蜘蛛 1種에서 各各 精巢를 摘出하여 DNA를 純粹分離하고 DNA鹽基를 定量分析하여 다음과 같은 結果를 얻었다.

(1) 家蠶(蛹體) 및 벳뚜기 類의 精巢 DNA의 A+T/G+C 比는 各各 1.72, 1.67로서 이 鹽基比는 게(蟹)精巢 DNA의 그것과 거의 같고 새우의 것과는 다르다.

(2) 蜘蛛精巢 DNA의 A+T/G+C比는 1.57로서 게(蟹)와 비슷하다.

(3) 昆虫 및 蜘蛛 精巢 DNA에서 methylcystosine을 發見못하였다.

Ⅰ. Introduction

Desoxyribonucleic acid (DNA) molecules that make up hereditary determinants must have at least two missions, e.g., genetic information and replication. These specificities of DNA molecules are attributed to their unique structure and sequence of four nucleotides possessing purine and pyrimidine bases such as adenine(A), guanine(G), cytosine(C) and thymine(T). The genetic code which is nucleotide sequence is translated into amino acid sequence of protein synthesis. Since that specific proteins not only form many of the structural cellular components but, more importantly, control as enzymes most of biosynthetic and metabolic events in living cells, the nucleotide composition of DNA is proved to be the basic information site presiding the specific biochemical events and structures of living organisms.

It is generally agreed that the content of DNA per cell is constant in the different tissues of same organism [1], as well as the proportions of its purine and pyrimidine bases. [2] But it can be expected the variability of base compositions among different orgamisms. In fact, their wide diversity was shown to exist in DNA of micro-organisms which present A+T/G+C ratio varying from 0.4 to 2.4 [2], where as DNA nucleotide compositions in micro-organisms varies widely, those of animals are generally believed to be similar. One of authors reported the base proportion of testes of mollusks and discussed its taxonomical significance. [3] We report here the result of DNA base analysis of testes of several insects and spider.

Ⅱ. Materials and methods

A silkworm, two sorts of locusts and a spider were used in this experiment. The testes were obtained from some twenty pupae just prior to hatching of slkiworms and several hundreds adults of

locusts and spider each at mating period. The testis of each species was pooled and DNA samples were prepared by the modification of the method described by Marmur. [4] Homogenates were made in Teflon homogenizer in the cold with saline-EDTA solution, followed by addition of sodium lauryl ulfate (SLS) solution to final concentration of 1%. After heating to 55°C for 10 min under constant agitation, suspensions were added by NaCl crystals to the final concentration of 1 M and agitated for 20 min, then centrifuged at 10,000g for 10min. The supernatant was deproteinized several times by Sevag's method [5] and crude DNA was obtained by successive addition of 3.0 M Na-acetate-0,001 M EDTA solution and isopropanol. Crude DNA was redissolved in dilute saline-citrate solution and subjcted to protein removal by Sevag's method and to precipitation with isopropanol. DNA thus obtained was again redissolved in dilute saline-EDTA and then treated with preheated RNase for the elimination of RNA contamination and DNA precipitated by iso-propanol was dried in usual manner.

Analysis of purine and pyrimidine bases: DNA bases were analyzed by Wyatt's method. [6] DNA samples were hydrolyzed in small sealed pyrex tubes with formic acid (98-100%) at 175°C for 40 min. Hydrolyzates were evaporated to dryness and residues were dissolved in 1.0N HCl, and these soultions were subjected to descending paper chromatography using Whatman No. I filter paper and HCl-isopropanol-H_2O (68:18:14) as developing solvent. The separated spots of purine and pyrimidine bases on dried paper chromatograms were detected employing UV lamp (2537Å) and cut out to be eluted with 0.1N HCl solution. Each eluate was subjected to the determination of optical absorbancy of bases at each maximum absorption as well as at 290mμ as internal reference. The amounts of each base were calculated as μ-moles using each molar extinction coefficient.

Ⅲ. Results

The analytical data of the testis DNA bases of three species of insects and one species of spider are summarized as follows.

Silkworm (*Bombyxmori*)

Bases	A(Adenine)	T(Thymine)	G(Guanine)	C(Cytosine)
micromoles.	0.721	0.713	0.405	0.423
corresponding to each	0.708	0.678	0.405	0.410
chromatographic spot×10	0.781	0.789	0.444	0.463
molecules of	39.87	31.52	17.90	18.70
bases (%).	32.16	30.80	18.40	18.62
	31.53	31.85	17.92	18.69
average of %	31.85	31.39	18.07	18.67

$\frac{\text{Purine}}{\text{Pyrimidine}}=0.99$ $\frac{A+T}{G+C}=1.72$ $\frac{G+C}{A+T}=0.58$

grasshopper (*Acrida lata Motschulsky*)

Bases	A(Adenine)	T(Thymine)	G(Guanine)	C(Cytosine)
micromoles.	1.487	1.423	0.840	0.835
corresponding to each	1.454	1.430	0.854	0.793
chromatographic spot×10	1.309	1.284	0.694	0.760
molecules of	32.43	31.03	18.32	18.21
bases (%).	32.09	31.56	18.84	17.50
	32.34	31.72	17.14	18.77
auerage of %	32.28	31.43	18.10	18.16

$\frac{\text{Purine}}{\text{Pyrimidine}}=1.02$ $\frac{A+T}{G+C}=1.75$ $\frac{G+C}{A+T}=0.569$

spider (*Tegenaria domesti Cerck*)

Bases	A(Adenine)	T(Thymine)	G(Guanine)	C(Cytosine)
micromoles	4.350	4.456	2.889	2.874
corresponding to each	4.602	4.561	2.961	2.900
chromatographic spot×10	4.894	4.853	3.027	2.973
molecules of	29.85	30.58	19.82	19.72
basls (%)	30.63	30.35	19.70	19.30
	31.07	30.81	19.22	18.87
average of %	30.51	30.58	19.58	19.29

$\frac{\text{Purine}}{\text{Pyrimidine}}=1.00$ $\frac{A+T}{G+C}=1.57$ $\frac{G+C}{A+T}=0.636$

locust (*Oxya velox Fabricius*)

Bases	A(Adenine)	T(Thymine)	G(Guanine)	C(Cytosine)
micromples	2.708	2.690	1.744	1.715
corresponding to each	2.642	2.690	1.672	1.668
chromatographic spot×10	3.016	3.052	1.872	1.854
molecules of	30.57	30.37	19.69	19.36
bases (%)	30.46	31.01	19.28	19.23
	30.79	31.16	19.11	18.92
average of %	30.60	30.84	19.36	19.17

$\frac{\text{Purine}}{\text{Pyrimidine}}=1.00$ $\frac{A+T}{G+C}=1.59$ $\frac{G+C}{A+T}=0.627$

The A+T/G+C ratios of testis DNA of silkworm (pupa), grasshoppers and spider are 1.72, 1.75-1.59 and 1.57 respectively.

According to the study of one of authors, the A+T/G+C ratios of the testis DNA of 4 species of crabs and lobsters each, are 1.7 and 2.0 respectively. [7] The *Insecta*, *Crustacea* (crabs, lobsters and shrimps) and *Arachnida* (spiders), are all included in Phylum *Arthropoda*. The base ratio of testis DNA of insects resembles that of crabs which is quite different from the base proportion of lobsters in spite of both belonging to crustaceans. So, the insects are closely related to the crabs from the view point of DNA base ratio. The A+T/G+C ratio of testis DNA of the spider is similar to those of the insects and crabs rather than lobsters.

Our present data suggests the additional evedence of the apprecial variation of DNA base composition with possible taxonomical significance in higher organisms other than micro-organisms and algae.

According to the data available in literatures, [8][9] the variations in DNA base proportion in higher animals and plants keep narrow range.

Ⅳ. Conclusion

Three species of insects and one species of spider were subjected to the base analysis of testis DNA. The following results are obtained.

(1) The A+T/G+C ratios of testis DNA of silkworm (pupa), grasshoppers and spider are 1.72, 1.75-1.59 and 1.57 respectively.

(2) The base proportion of testis DNA of insects is quite similar to that of crabs but different from that of lobsters.

(3) The A+T/G+C ratio of testis DNA of spider resembles those of insects and crabs rather than lobsters or shrimps.

(4) No methylcytosine was found in testis DNA of the insects and spider.

Ⅴ. References

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