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DNA SEQUENCE ORGANIZATION IN TWO ODONATE SPECIES, *CALOPTERYX SPLENDENS* (HARRIS) AND *AESHNA COERULEA* (STRÖM) (ZYGOPTERA: CALOPTERYGIDAE; ANISOPTERA: AESHNIDAE)*

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The organization of the repeated and single-copy DNA sequences in the genomes of 2 odon. spp. from different suborders was studied. Three types of experiments were performed, viz. (1) comparison of the reassociation kinetics of short (200 nucleotides) and long (700-900 nucleotides) DNA fragments, (2) measurement of the hyperchromicity of the reassociated repeated sequences as a function of fragment length, and (3) sizing S_1 nuclease-resistant reassociated repeated sequences. The data indicate that in the genomes of dragonflies only a part of repeats are interspersed with single-copy sequences in a "*Xenopus* pattern" and that mostly they are organized in a "*Drosophila* pattern". Consequently, one can hardly speak of a definite type of genome organization; it may differ depending on the genome size.

INTRODUCTION

It has been demonstrated that a varying part of the eukaryotic genome is arranged as alternating repeated and single copy DNA sequences. In a short-period interspersion pattern ("*Xenopus*-pattern"), short repeated sequences (300 base pairs) alternate with longer single copy ones (800-1200 bp). This pattern turned out to be typical of most of the eukaryotic species studied. A different,

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so-called long-period interspersion pattern ("*Drosophila*-pattern"), has been observed among small genomes of insects. The genomes of such insect species appear to consist of repeats several thousand nucleotides long interspersed with very long single copy sequences. Genomes lacking a short-period interspersion pattern are characteristic of some other eukaryotic organisms (SCHMIDTKE & EPPLEN, 1980).

The existence of the two patterns of DNA sequence organization raises some questions about the functional organization and evolution of eukaryotic genomes, since the two patterns may be encountered within a group of a low taxonomic rank, e.g., within the order Diptera. It is assumed that repetitive DNA sequence organization has significant implications for the functional organization of the genome (DAVIDSON & BRITTEN, 1973). It is difficult to comprehend how the two patterns of structural and functional organization of genomes could have evolved in phylogenetically closely related species.

The absence of short-period interspersion appears to correlate with small genome size. It has been suggested that the long-period pattern could have evolved from the short-period pattern by a selective loss of specific DNA segments (CRAIN et al., 1976b). As until now this view has received little experimental support, an alternative mechanism of evolution from a long to a short-period pattern cannot be excluded.

Taking into account this uncertainty we have examined the DNA sequence organization of two species of Odonata, an order which from an evolutionary point of view, is quite distinct from those which have been studied in this respect among insects (Diptera, Lepidoptera).

MATERIAL AND METHODS

Preparation of DNA

DNA was isolated from ethanol-fixed adult animals by a combination of the method of ARRIGHI et al. (1968) and the MUP method (BRITTEN et al., 1974).

DNA fragmentation and sizing

Short DNA fragments were prepared either by sonication or by shearing DNA in a homogenizer. For some experiments DNA fragments were fractionated on a Sepharose CL-4B column in 0.3 M NaCl + 0.1 M NaOH, pH 12.5. Sizing of the DNA fragments was performed according to STUDIER (1965). Short DNA fragments were about 200 nucleotides, and longer fragments, 700 and 900 nucleotides long. Fragments from free volume CL-4B column fractions were 1400 and 1600 nucleotides long.

DNA reassociation

The DNA samples were reassociated in either 0.08 M phosphate buffer (PB) at 57° C, or 0.12 M PB at 60° C, or 0.4 M PB at 66° C. Double-stranded molecules were separated from single-stranded ones by hydroxy-apatite (HAP) chromatography under standard conditions (BRITTEN et al., 1974). In several experiments, the double-stranded fragments were eluted from HAP by raising the column temperature to 95° C in 5° C increments.

S_1-nuclease treatment of reassociated DNA

It was performed as recommended by BRITTEN et al. (1976).

Sizing of S_1-resistant duplexes

The S_1-resistant duplexes were chromatographed on a Sepharose CL-4B column (0.6 x 50 cm) in 0.12 M PB, pH 6.8.

Hyperchromicity measurements

These were performed, using an SP-800 (Pye Unicam, England) spectrophotometer equipped with an outer recorder for scale expansion. All absorbance values were corrected for solvent thermal expansion.

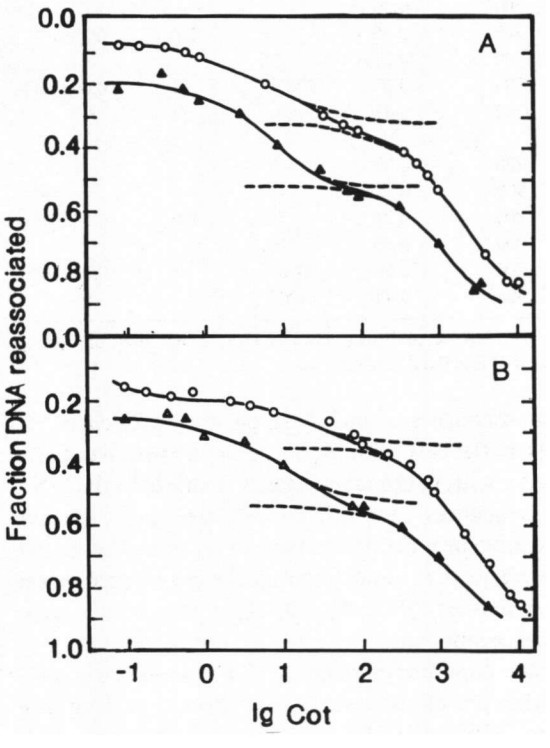

Fig. 1. Reassociation kinetics of short and long DNA fragments of two dragonflies, *Aeshna coerulea* (A) and *Calopteryx splendens* (B). — Open circles (○): short DNA fragments (200 nucleotides); — Triangles (▲): long DNA fragments (900 nucleotides for A and 700 nucleotides for B). The dashed lines represent the calculated second-order components with the parameters listed in Table I.

RESULTS

BASE COMPOSITION

The base composition was determined from Tm of DNA fragments 700 and 900 nucleotides long corrected for the effect of fragment length: Δ Tm = 650/L (BRITTEN et al., 1974) and was found to be 40.5% GC for *Aeshna coerulea* and 44.9% GC for *Calopteryx splendens*.

REASSOCIATION KINETICS OF DNA OF DIFFERENT FRAGMENT LENGTH

The results of reassociation kinetics analysis are shown in Figure 1. It can be seen that in the case of short (200 bp) fragments the reassociation curves of both species consist of three kinetic components. The sequences of the first of them reassociate at $C_0t < 10^{-1}$. A part of this fraction is evidently represented by foldback DNA, the bulk of it, however, consists of highly repetitive sequences. The DNA of the second component reassociates at 10^{-1} to 10^{2} C_0t and consists of moderately repetitive sequences. The DNA of the slowest component

Table I
Components of dragonfly DNAs estimated from reassociation kinetics*

Component	Fragment length (nucleotides)	Fraction of genome	C_0t ½	Repetition frequency	Complexity (daltons)
Calopteryx splendens					
Zero time + fast reassociating	200	0.18	—	—	—
	700	0.26	—	—	—
Middle repetitive	200	0.15	7.5	250	0.58×10^9
	700	0.27	7.5	—	—
Single-copy	200	0.63	1900	1	0.60×10^{12}
	700	0.43	1180	1	—
Aeshna coerulea					
Zero time + fast reassociating	200	0.09	—	—	—
	900	0.18	—	—	—
Middle repetitive	200	0.24	5.2	380	0.63×10^9
	900	0.36	5.2	—	—
Single-copy	200	0.63	2000	1	0.64×10^{12}
	900	0.42	1100	1	—

* Experimental data were described with two second order components, using equation $C/C_0 = 1/1+K_2C_0t$. Fraction DNA unreassociated was fixed at level 0.04.

reassociates at 10^2 to 10^4 C_0t and consists of mostly single copy sequences.

The curves in Figure 1 represent the best solution of the experimental data for the two second-order components with parameters listed in Table I. In the DNA of both species, single copy sequences amount to 63%, whereas there are significant differences in the relative proportions of various classes of repetitive sequences. In the DNA of *A. coerulea* the quantity of middle repetitive sequences is 10% greater than in the *C. splendens* DNA. The DNAs of these species also differ in the content of the fastest component.

The C_0t ½ values of the single copy components for these species are 1900 and 2000 for total DNA. With the correction factor for the effect of the fragment length on the reassociation rate (WETMUR & DAVIDSON, 1968) and C_0t ½ value = 4 for *E. coli* DNA (BRITTEN & KOHNE, 1968) the genome sizes of *A. coerulea* and *C. splendens* are 1×10^{12} daltons (1.6 pg) and 0.95×10^{12} daltons (1.5 pg), respectively. These values are lower than those of species of Orthoptera (WILMOR & BROWN, 1975) and higher than those of Diptera.

The data on the reassociation kinetics of longer (700 and 900 bp) DNA fragments are also shown in Figure 1. As in the case of short fragments, the reassociation curves for fragments 700 and 900 nucleotides long consists of three kinetic components. The slowest of these components is evidently represented by single copy sequences. The kinetic parameters for two second-order components are listed in Table I. Fragments 700 bp long, containing only single copy sequences, are expected to reassociate with C_0t ½ = $1014/1900\sqrt{200/700}$. This

value is in good agreement with the experimental data listed in Table I. The same is true of 900 bp fragments. Therefore we may conclude that in the DNA of both dragonfly species about 40% of 700 and 900 bp fragments contain only single copy sequences.

With longer DNA fragments the relative content of the fast component ($C_0t < 0.1$) increased from 18 to 25% of the DNA for *C. splendens* and from 9 to 18% of the DNA for *A. coerulea*. The corresponding changes of the slow component were from 15 to 27% for *C. splendens* and from 24 to 36% for *A. coerulea*. The total increase in both cases (about 20%) corresponds to one third of the fraction of the single copy component (at the fragment length of 200 nucleotides). From these observations it follows that in long fragments one third of single copy sequences reassociates at the same C_0t values as repetitive sequences do. Thus these data imply that at least 20% of single copy sequences alternate with repeated sequences within 700-900 nucleotide long fragments.

HYPERCHROMICITY OF REASSOCIATED REPEATED DNA

When both repetitive and single copy sequences are located within the same DNA fragment, it remains partially unpaired after reassociation to low C_0t values. These partial duplexes have a lower hyperchromicity than complete duplexes. The fall in hyperchromicity will correlate with the increase in the length of the fragments and with the amount of the DNA arranged in a short interspersion pattern (GRAHAM et al., 1974; GOLDBERG et al., 1975).

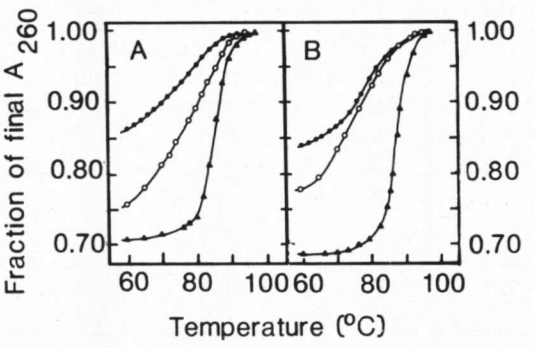

Fig. 2. Optical melting profiles of DNA fragments of various length after reassociation to repetitive Cot values, compared to those of native DNA: (A) *Aeshna coerulea*, (B) *Calopteryx splendens*. — Triangles (▲): native DNA fragments (700-900 nucleotides); — open circles (○): short (200 nucleotides) DNA fragments after reassociation to Cot 70; — closed circles (●): long (1400-1600 nucleotides) DNA fragments after reassociation to Cot 20.

In order to measure the hyperchromicity of the reassociated repeated sequences as a function of fragment length, the DNA fragments were reassociated to low C_0t values, duplexes were collected on hydroxyapatite, dialyzed against 0.12 M PB and melted. The data obtained are given in Figure 2. It can be seen that hyperchromicity decreases with the increase of the DNA fragment length. This observation is consistent with the view that in the

fractions of reassociated fragments there exists a significant portion of single copy sequences linked with relatively short repetitive sequences.

On the basis of the ratio between the hyperchromicity of the reassociated repetitive DNA and that of native DNA, the average length of the duplex regions in the reassociated DNA fraction was calculated (Tab. II). A correction factor of 0.025 (BRITTEN et al., 1974) was introduced for the hyperchromicity of single-stranded regions. Short fragments of DNA of both dragonfly species reassociated to repetitive C_0t values exhibit 69-72% of the hyperchromicity of native DNA. The average length of the duplex regions in this case is 140 bp. These values are in good agreement with theoretical ones (WETMUR & DAVIDSON, 1968). Thus these data indicate that short DNA fragments, reassociated to repetitive C_0t values have very few, if any, single copy sequences linked to them. We may conclude that the length of such repetitive sequences in the genomes of the dragonfly studied is at least 200 nucleotides.

Table II

Hyperchromicity of reassociated repetitive DNA as a function of the fragment length

Fragment length (nucleotides)	C_0t	Tm(° C)	Hyperchromicity (H)	H reassociated / H native	Average duplex length (nucleotides)
Calopteryx splendens					
200	70	76.5	0.225	0.63	130
700	70	78.0	0.202	0.56	390
1600	20	78.5	0.163	0.43	690
700	native	86.8	0.318	1.00	700
Aeshna coerulea					
200	70	78.2	0.236	0.72	140
900	70	81.0	0.194	0.58	520
1400	20	76.5	0.137	0.38	530
900	native	85.2	0.292	1.00	900

Hyperchromicity was calculated as (A_{260} 95° C — A_{260} 60° C)/A_{260} 95° C. The absorbance values were corrected for thermal expansion of the solvent. The average duplex length was calculated relative to the hyperchromicity of the native DNA after correction for single-stranded collapse between 60° C and 95° C, using a factor of 0.025 (BRITTEN et al., 1974).

Longer DNA fragments (700 and 900 bp), after reassociation to repetitive C_0t values, exhibit 56-58% of the hyperchromicity of native DNA. The average length of the duplexed regions in this case is 390 and 520 bp. It is evident that a part of the 700 and 900 bp DNA fragments, reassociated to repetitive C_0t values, carry single copy sequences linked to the reassociated repeats. Otherwise the hyperchromicity values and the average length of the duplexed regions would have been much higher. The hyperchromicity of 1400 and 1600 bp DNA fragments, reassociated

to repetitive C_0t values, is only 38-43% of that of native DNA and the average duplex length in this case is 530 and 690 bp. These values are significantly lower than those that could be expected for the DNA fragments without alternation of repetitive and single copy sequences over a distance of less than 1400-1600 nucleotides. Indeed, the reassociated middle repetitive DNA of the *Drosophila* genome with no detectable short interspersed repeats has the hyperchromicity value of about 79% of that of native DNA fragments 1660 nucleotides long (CRAIN et al., 1976a).

All in all, the results of the measurement of the hyperchromicity of reassociated repetitive DNA as a function of the fragment length suggest that in a part of the genomes of both dragonfly species short (700-900 bp) repetitive sequences alternate with longer single copy sequences.

SIZING OF S_1-RESISTANT FRAGMENTS OF REASSOCIATED REPETITIVE SEQUENCES

In order to determine the lengths of short interspersed repeats, long DNA fragments (1400 and 1600 bp) were reassociated to C_0t 20 and then digested with S_1 nuclease to DIG = 0.75 (BRITTEN et al., 1976). The S_1-resistant duplexes were collected on hydroxyapatite (23-24% for both species), eluted with 0.4 M PB and further chromatographed on a calibrated Sepharose CL-4B column (0.6x50 cm).

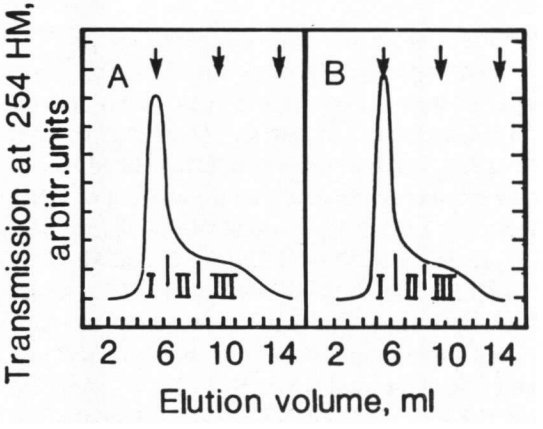

Fig. 3. Sepharose CL-4B column profiles of S_1-resistant repeated sequences after reassociation to Cot 20: (A) *Aeshna coerulea*, (B) *Calopteryx splendens*. — Arrows on the top indicate a position of markers of known length (left to right); 1200 nucleotides and more; 260 nucleotides; mononucleotides (ATP). — S_1-resistant material was collected into three fractions, as indicated on elution profiles.

The elution profiles of the S_1-resistant duplexes from the Sepharose CL-4B column are shown in Figure 3. The eluted duplexes were subdivided into three fractions (I, II, III) shown in Figure 3. Fraction I corresponds to the excluded duplexes more than 1000-1200 nucleotides long and fractions II and III, to the fractionated duplexes less than 1200-1000 bp. It can be seen that the mode of the third fraction coincides with the position of the 260 bp marker. It implies that a part of the S_1-resistant duplexes is formed by short, (~ 300

bp) sequences. The S_1-resistant duplexes of fractions I, II and III constitute 6.4, 6.9 and 9.6% respectively of the total DNA of *A. coerulea* and 13.0, 5.4 and 5.6% of that of *C. splendens.*

In order to confirm that the single-stranded "tails" in the S_1-resistant duplexes are not long, the DNA from each fraction was concentrated on hydroxyapatite and the melting properties of the S_1 resistant fractions of the *A. coerulea* DNA were determined. The hyperchromicity values for both the excluded fraction and fraction II are similar to those for native DNA, whereas the hyperchromicity values for short repeats are significantly lower. As no significant increase in absorbance in the range of 20° to 60° C was observed, the data should be taken to mean the completeness of digestion of single-stranded regions in the reassociated fraction of long fragments.

The Tm value of the excluded duplexes of *A. coerulea* in 0.12 M PB is 82.5° C, i.e., 3° C below that of its native DNA. The difference of 1-3° C was reported for some other species (GOLDBERG et al., 1975; CRAIN et al., 1976b).

The difference in Tm of native DNA and short repeats in the DNA of *A. coerulea,* is about 8.5° C, as in many other eukaryotic DNAs. It may reflect a greater intragenomic divergence of short repeats compared to long ones.

The results obtained suggest that part of the repetitive DNA of *Calopteryx splendens* and *Aeshna coerulea* is represented by short repeats as in many other eukaryotic species studied. However, the amount of short repeats in the DNA of dragonflies is smaller than in DNAs of other animal species with a similar pattern of genome organization.

The same conclusion can be drawn from the hydroxyapatite temperature elution profiles of the reassociated repeated DNA. Short (200 bp) DNA fragments of both dragonfly species were reassociated to $C_0t = 100$ and the duplexes were collected on hydroxyapatite at 60° C. Then the column temperature was raised step-wise to 95° C and the amount of DNA eluted at each step was measured. Results of this estimation indicate that the amount of DNA eluted between 60 and 70° C is relatively low; 10.5% of total DNA for *A. coerulea* and 7.5% for *C. splendens.* The amounts of DNA in 70-80° C and 80-95° C fractions are, respectively, 16.7 and 13.9% of the total DNA of *A. coerulea* and 16.7 and 16.5% of that of *C. splendens.* In similar experiments with *Strongylocentrotus purpuratus* DNA the amounts of the corresponding fractions were found to constitute 11, 19 and 12% of the total DNA (BRITTEN et al., 1976) and the DNA of *S. intermedius,* 8.5, 9.0 and 3.0% (PETROV & POLTARAUS, 1980). It has been shown that the DNA of the 60-70° C fraction thus obtained is represented mainly by short repeats, whereas the DNA of the 80-95° C fraction consists of long repeats (BRITTEN et al., 1976; PETROV & POLTARAUS, 1980).

DISCUSSION

The results of the experiments described can be summarized as follows. In both of the dragonfly species studied, about 30% of DNA is represented by repeated nucleotide sequences. About one third of repetitive DNA (300 bp long) alternates with single copy sequences of a much greater length. The other part is represented by longer (1400-1600 bp) fragments which are clustered or organized into a long--period interspersion pattern. The single-copy sequences constitute about 60% of the DNA of both species. Part of these sequences alternate with short repeats within 1400-1600 bp fragments, whereas the rest of them are organized into much longer sequences. It remains unknown whether there are single-copy sequences not spaced by repetitive sequences at very long distances.

Although the genome organization of both species studied is similar, some differences are obvious. The DNAs of *Aeshna coerulea* and *Calopteryx splendens* differ in their base composition, in the proportion of sequences of various repetition frequency, and in the ratio between long and short repeated sequences. At the moment it is difficult to correlate these differences with the functional organization of the genomes and with the general mode of their evolution.

To understand the genome evolution, the similarity between the sequence--pattern organization of DNAs of the two dragonfly species belonging to different suborders may be of greater significance than quantitative differences. In fact, it may be suggested that genomes of all other dragonfly species are organized similarly to those of the two species studied, although it cannot be ruled out that some dragonflies will be found to lack a short interspersion pattern. It has been reported that insects with genomes smaller than 0.7 pg have no short-period interspersion pattern, whereas all other arthropod species display the short--period interspersion pattern. Among other animal branches, the short-period pattern was not observed in some Nematoda species and birds (SCHMIDTKE & EPPLEN, 1980). The same pattern is inherent in the DNA of pearl millet, *Pennisetum americanum* (WIMPEE & RAWSON, 1979). As a rule, the genomes of these species are smaller than 1 pg. Birds, which have been shown to lack a short-period pattern have genomes ranging from 1 to 1.65 pg. These values are the lowest in the Sauropsida branch. Thus in all the species studied, the lack of short-period patterns appears to correlate with small genome size. This may suggest that a long-period pattern could have evolved from a short-period pattern by selective deletion of specific DNA segments (CRAIN et al., 1976b), although the opposite direction of evolution cannot be ruled out. When discussing this problem, we must remember that specialized species of various eukaryotic lineages have smaller genomes than generalized ones (HINEGARDNER, 1976). In the light of the ideas of NAGL (1978) concerning the mechanisms of genome evolution, the former alternative seems more realistic.

It is noteworthy that two different patterns of genome organization are observed within Diptera, a highly organized and fast evolving group of Insecta. No short-period pattern has been found in one of the species from another evolutionary progressive group of insects, Hymenoptera (CRAIN et al., 1976b). The 5.6 pg genome of the most primitive *Thermobya domestica* (Thysanura) has a short-period interspersion pattern (FRENCH & MANNING, 1980). The relatively small 1.5-1.6 pg genomes of two dragonfly species belonging to one of the primitive groups of living insects are only partially organized into a short period pattern.

All these observations thus support the view that the lack of the short-period pattern correlates with the small genome size and with the level of morphological specialization. It is clear, however, that these findings are insufficient for understanding the evolutionary history and origin of short- and long-period interspersion patterns. Therefore it is necessary to obtain more data on the distribution of genome organization patterns among the groups of Insecta of different evolutionary history.

Our preliminary data on DNA reassociation kinetics of other dragonfly species show a great variety of genome sizes among species studied. Thus, genome sizes of the gomphid dragonflies are about 0.4 pg. It may be expected that genomes of these species lack a short-period interspersion pattern. At the moment we are studying this problem.

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