VARIABILITY OF THE DNA CONTENT IN FIVE ORTHOPTERAN SPECIES

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INTRODUCTION

DNA content is now known from direct measurements for close to a thousand species of animals and plants and from indirect measurements, such as calculations from chromosome or nuclear size, for many additional species. These amounts in free-living organisms range from 0.007 pg. for an average bacterium to 100 pg. or more for the haploid amount of DNA in some plants and salamanders (SPARROW *et al.* 1972).

The first relationship between DNA content and the evolutionary process comes from MIRSKY and RIS's works (1951). They suggest three relationships between DNA content and evolution: i) There might be an increase in DNA content, going from the lower invertebrates to the higher invertebrates; ii) related organisms, such as members of the same family, tend to have similar amounts of DNA; iii) evolution of the land vertebrates may have been accompanied by DNA decrease.

In Orthoptera, these measurements have revealed some evolutive paths of great interest: FOX (1970), and WILLMORE and BROWN (1975), established values for the DNA content in different species. JOHN and HEWITT (1966) found that significant differences in DNA content exist both between species within the same chromosome group and between member species of the $2n = 17$ and $2n = 23$ groups.

The species in the sub-family Truxalinae used in this work present karyotypes ($2n = 17$ in male individuals) with few variations among them. They have 6 metacentric chromosomes and 11 acrocentrics including the X-chromosome. The sex chromosome mechanism is an X0/XX type. All these

homologies induced us to measure the DNA content trying to uncover the possible variations ocurring among the studied species. On the other hand, a DNA measurement of the species *Heteracris adspersus* was used when trying to establish any relationship between the forms $2n = 17$ and $2n = 23$.

MATERIAL AND METHODS

Adult males from natural populations of *Chorthippus longicornis*, *Ch. vagans*, *Ch. brunneus*, *Gomphocerus sibiricus* and *Heteracris adspersus* were used. Testes were fixed in 3:1 absolute ethanol acetic acid. Feulgen stain was developed as usual: 1N HCl hydrolysis was undertaken for 8 min., and after Feulgen reaction the material was washed in a solution of 5 ml HCl: 5 ml Na-metabisulphite: 10 ml H_2O, then air dried and mounted. This treatment was applied simultaneously to a squashed root of *Allium cepa* on the same slide, which was used as a control. The standard is taken to be $2C = 33 \times 10^{-12}$ gr.

The DNA values of 1C spermatid and 2C nuclei from the different species and *A. cepa* respectively, were determined densitometrically using the Vickers M-85 integrating microdensitometer, and the total amount of stain per nucleus was measured at a wavelenght of 550 nm for 36 nuclei from three individuals of each species. Different cells were measured at random, and in the same stage of development.

RESULTS

In the species of the genus *Chorthippus*, as in a majority of the genera in the sub-family Truxalinae, the mitotic chromosome complement of the male includes seventeen members. Sixteen of these are autosomes and can be grouped into eight homologous pairs, three of them are metacentric and the remainder acrocentric; the seventeenth chromosome is the umpaired X or sex chromosome (JOHN and LEWIS 1965). The same pattern of chromosome distribution is obtained with the Pyrenean species *G. sibiricus*. *H. adspersus* presents a diploid number $2n = 22 + X$ being all the chromosomes acrocentrics.

The distribution of DNA values for the four species of the Truxalinae group shows a similar range (7.5 - 11.5); however, the species *H. adspersus* presents lower values and they are comprised in the range (5.25 - 7.75) (see Fig. 1). The 1C contents fall into the range established for the Ortopteroid insects ($6–14 \times 10^{-12}$gr.) in both groups of evolution. In the same way, the results obtained for *Ch. brunneus* agree with earlier estimates (9.46×10^{-12}gr., WILLMORE and BROWN 1975) (Table 1).

As superficially all four karyotypes appear very similar in morphology, a nested analysis of variance to compare the DNA measures given in Fig. 1

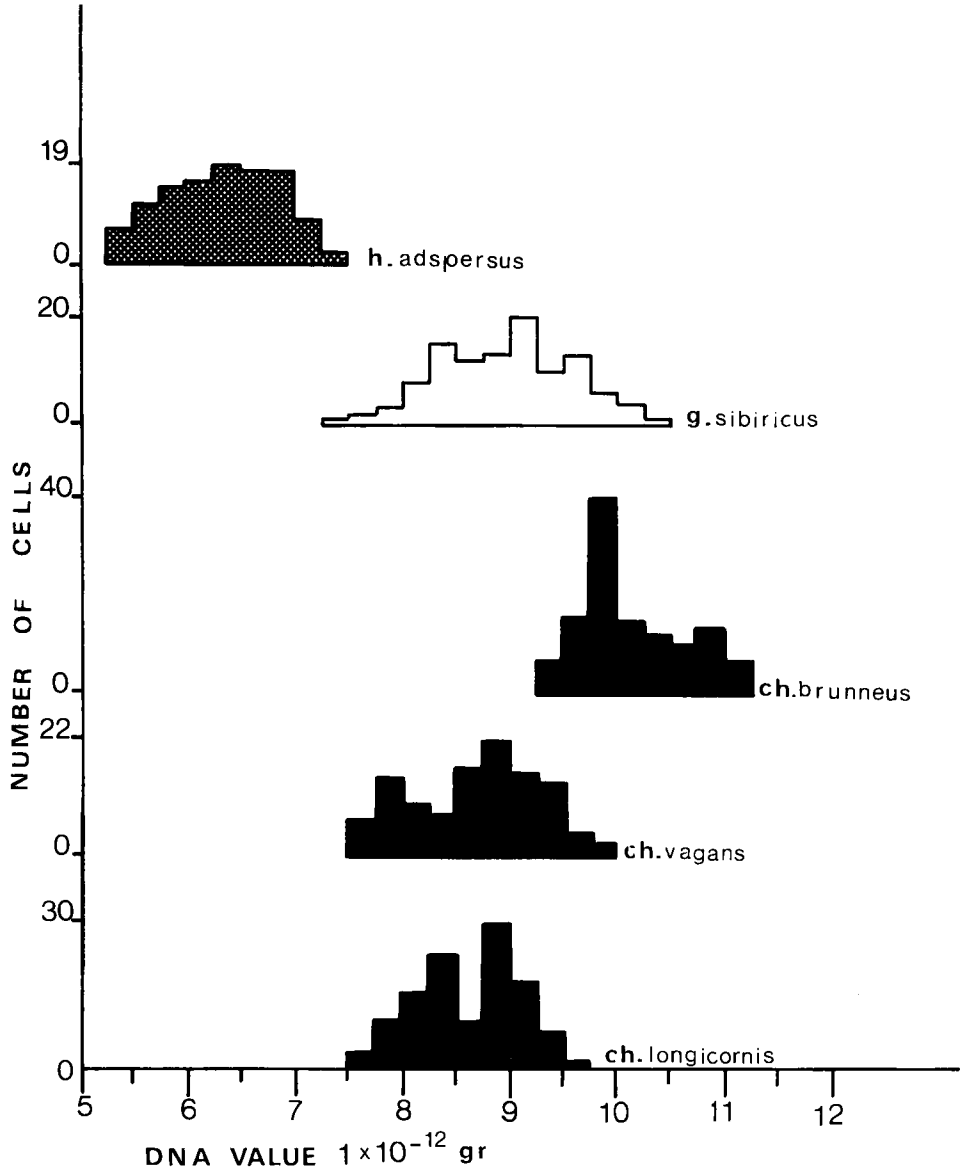

Fig. 1. — A comparison of the DNA values of the five species.

was used. As significant interspecific differences were obtained, the analysis was continued omitting the values of different species (Table 2). The first analysis was performed using the five species and there is a significant difference in DNA value. (Table 2a). If the analysis was repeated, now

TABLE 1

DNA content and chromosome number in the five studied species.

Species	Chromosome Number	DNA value $x10^{-12}$ gr.	$6n$-1	Number of measurements
Ch. longicornis	17	8.58	0.0615	108
Ch. vangans	17	8.68	0.3364	108
Ch. brunneus	17	10.15	0.2401	108
G. sibiricus	17	8.95	0.3969	108
H. adspersus	23	6.34	0.2601	108

TABLE 2

Analysis of variance of DNA values for the different species shown in Fig. 1. Item (a) between the five species. Item (b) omitting the H.adspersus values. Item (c) within species of Genus Chorthippus. Item (d) between the species Ch.longicornis, Ch.vagans and G. sibiricus. s: between species. i/s: between individuals within species. i: within individuals.

Item	dF	SS	MS	VR	P 0.005
(a)					
1. s	4	818.51	204.62	581.32	Sig.
2. i/s	10	3.52	0.35	1.24	Not. sig.
3. i	525	148.31	0.28		
4. Totals.	539	970.34			
(b)					
1. s	3	163.49	54.49	51.57	Sig.
2. i/s	8	3.17	0.39	1.36	Not. sig.
3. i	420	121.77	0.28		
4. Totals.	431	288.43			
(c)					
1. s	2	160.08	80.04	163.91	Sig.
2. i/s	6	2.93	0.48	1.93	Not. sig.
3. i	315	79.68	0.25		
4. Totals.	323	242.69			
(d)					
1. s	2	6.43	3.21	8.11	Sig.
2. i/s	6	2.38	0.39	1.26	Not. sig.
3. i	315	98.29	0.31		
4. Totals.	323	107.01			

omitting *H. adspersus,* there are also significant differences, but these differences are now within the Truxalinae group. (Table 2b). A third analysis was made with the species within the genus *Chorthippus.* In this case and being at the genus level, significant differences were obtained, too. (Table 2c).

This difference disappears if the *Ch. brunneus* values are omitted. (Table 3). If the analysis of this interspecific variation is repeated with *Ch. longicornis*, *Ch. vagans* and *G. sibiricus* data, the values are still significantly different. (Table 2d).

TABLE 3
A comparison of the DNA valus for the species Ch. longicornis and Ch. vagans.

Species	$\bar{X}$	V_x	n	t_{214}	$P_{0.01}$
Ch. longicornis	8.61	0.2209	108	0.974	Not. sig.
Ch. vagans	8.68	0.3364	108		

DISCUSSION

Increases and decreases in the amount of cellular DNA have played a major role in the evolution of organisms, and appreciably different amounts of DNA may be found in species belonging to the same family and with the same number of chromosomes (ULLERICH 1966). So a DNA comparison is capable of reflecting evolutionary changes that may pass undertaken from a simple comparison of chromosome morphology.

From the results obtained in the present study we can see that: a) The mean DNA content of 1C spermatic nuclei are comprised in the range established for Orthopteroid insects; b) *Ch. vagans* and *Ch. longicornis* present highly similar DNA amounts; c) between the three species of the genus *Chorthippus* analized, the DNA content for the species *Ch. brunneus* is the highest; d) *H. adspersus* presents the lowest value which agrees with the pattern obtained for other $2n = 23$ forms (JOHN and HEWITT 1966); e) significant differences appear when *G. sibiricus* is compared with *Ch. longicornis* and *Ch. vagans*, but all of them present very close DNA amounts.

The differences found in the DNA content, do not only appear between different genera, but they can also appear within the specific level. The differences obtained at this level have a great interest as they can reflect differences in the DNA content of very related species. Alhough there is no direct evidence on the origin of these variations in the DNA content, the process of duplication seems to play, in the concrete case of Orthoptera, an important role in the evolution of different species (WHITE 1978). There are many quite commun polymorphisms within this group, such as heterochromatic segments attached to autosomic bivalents which seem to be arisen as a massive tandem duplication of certain chromosome regions (JOHN 1973; HEWITT 1979). These segments, in some cases, present a very great size

and may duplicate the normal size of the bivalent (GOSALVEZ *et al.*, in preparation); this fact would implicate an add of chromosome material quite great compared to the total size of the genome. REES *et al.* (1978) found that males of the northern race of *Cryptobothrus chysophorus* contain about 20% more nuclear DNA than males from the southern race, and they suggest that the DNA difference may be explained by supernumerary segments within chromosomes.

At the single gene level there is the amino-acid squence analysis showing that proteins such as the digestive enzymes trypsin, chymotripsin and elastase are the products of gene duplication (SHOTTON and HARTLEY 1970). In the same way, the hemoglobins have also arisen by gene duplication (ZUCKERKANDL 1965). The phenomenon of polyploidization, which is present in many organisms, but particularly in plants, may play a similar role in the process of speciation. So there is no reason to believe that similar events are not now ocurring in contemporary organisms.

In the different taxa here studied, the process of speciation does not seem to have been developed at the genus level as a taxonomic group; this process has been developed at specific level. This idea is based on the data obtained for the species *Ch. longicornis*, *Ch. vagans* and *G. sibiricus*. The DNA values for *G. sibiricus* compared with those obtained for *Ch. longicornis* and *Ch. vagans*, show significant differences, but these differences are higher if the species *Ch. brunneus* is included in the analysis. This fact indicates that members of many groups have been able to prosper with significantly more DNA than their relatives, and members of distant groups may mantain similar DNA amounts. In other cases such as *Thyanta* species (SCHRADER and HUGES-SCHRADER 1956) and *Banasa* species (SCHRADER and HUGES-SCHRADER 1958) a similar condition has been observed.

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SUMMARY

The DNA content of five Orthopteran species from the genus *Chorthippus, Gomphocerus* and *Heteracris* was calculated using an integrating microdensitometer. Results show that values are between $6\text{–}14\times10^{-12}$ gr. *Heteracris adspersus*, which is the unique analyzed species with $2n = 23$ show the lowest value; the rest of the species show values of a relatively similitude. Nevertheless, differences exist between genera and between species within genus when an analysis of variance test was applied. The possible relationship with duplication processes and the obtained results are discussed.