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Genome size in *Tribolium* flour-beetles: inter- and intraspecific variation

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Summary

Eight species of *Tribolium* and the related species *Alphitobius diaperinus* have been microdensitometrically analysed by measuring the nuclear content (1C value) of their Feulgen-stained spermatids. The range of mean genome sizes goes from 0·157 pg in *T. audax* to 0·388 pg in *T. brevicornis*, including five significantly different groups of values. Also, in all but one species of *Tribolium* significant intraspecific heterogeneity of genome size was found. The resemblances in genome size are not generally correlated with genetic homologies among species, but there is a highly significant correlation between genome size and spermatid size.

1. Introduction

Much genetic knowledge has been accumulated in recent years on the *Tribolium* flour-beetles (Sokoloff, 1966, 1972, 1974, 1977), which clearly exceeds that on any other species of Coleoptera. But nothing has been published on the nuclear DNA content of this group except for some preliminary results on *T. castaneum* (Juan & Petitpierre, 1989). The genome size is a species-specific character upon which several evolutionary assumptions have been proposed and are still a matter of debate (for general reviews see Cavalier-Smith (1985) and John & Miklos (1988)). Intraspecific variation of genome size was not considered in the first analyses. Recent papers have claimed its existence in animals, e.g. mosquitoes (Rao & Rai, 1987; Ferrari & Rai, 1989), fresh-water fishes (Gold & Amemiya, 1987; Ragland & Gold, 1989) and *Thomomys* rodents (Sherwood & Patton, 1982), and in plants, e.g. *Scilla* (Greilhuber & Speta, 1985), *Microseris* (Price *et al.* 1980) and *Zea mays* (Rayburn *et al.* 1985; Laurie & Bennett, 1985). This paper describes a study of both intraspecific and interspecific genome size variation in eight species of *Tribolium* and *Alphitobius diaperinus*, a related species belonging to the same tribe (Ulomini).

2. Materials and methods

The sources of the species studied were as follows: *Tribolium audax* Halstead, *T. brevicornis* Lec., *T. confusum* Duval, *T. freemani* Hinton and *T. madens* (Charp.) were obtained from the *Tribolium* Stock Center at California State University, San Bernardino (California, U.S.A.). *Tribolium anaphe* Hinton, *T. destructor* Uytten. and *Alphitobius diaperinus* Panzer from ADAS Central Science Laboratory (Slough, England).

Relative DNA content was measured in Feulgen-stained spermatids of 10 individuals of each *Tribolium* species, and on 5 individuals of *Alphitobius diaperinus*. Feulgen staining was made by the procedure of Juan & Petitpierre (1989). Briefly, squashed male gonads were fixed in 10% formalin for 10 min, hydrolysed in 5 N-HCl for 45 min. at 22 °C and stained with Schiff's reagent for 2 hours at the same temperature. The slides were then dehydrated, cleared in xylol and mounted in Depex (Gurr's). This treatment gave maximal staining in a series of control experiments using different times, temperatures and concentrations of HCl.

Twenty round and homogeneously stained spermatids were measured per individual with a Leitz MPV cytospectrophotometer, and standardized as a percentage of the mean transmission value of *Tribolium castaneum* (Consejo strain) treated and stained under identical conditions, giving rise to a total of 200 measurements per species. The mean genome size of *T. castaneum* was calibrated by comparison with that of the dermestid beetle *Dermestes maculatus*, with a C value of 1·129 pg (Rees *et al.* 1976). 100 spermatids, twenty per individual, were measured in both species by an identical series of Feulgen staining treatments to

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set up the absolute DNA content of *T. castaneum*. Moreover, the size of each checked spermatid was calculated from the diameter measured at 2000x by an ocular micrometer. Then the linear scale units were transformed into μm^2 to obtain the spermatid area for a possible correlation with the DNA content.

Sample distributions of genome size were used for Student-Newman-Keuls (SNK) multirange test and analysis of variance (ANOVA). The "genome size distances", GSD_{kl} (minimum genome size difference between individuals of two species) and GSD_k (average genome size difference between individuals of a species) proposed by Gold & Amemiya (1987) were also calculated.

3. Results

First, we measured the mean light extinctions of spermatids from *Dermestes maculatus* and *Tribolium castaneum*, given in arbitrary units as 25·963 ± 0·237 and 4·789 ± 0·058, respectively. This yielded a mean C value for *T. castaneum* of 0·208 ± 0·002 pg, which was taken as standard for calibrating those of the other species.

Table 1. *Main statistical parameters of average DNA spermatid values per species*

Species	n[a]	Mean ± S.E.[b] (pg)	Mean range
Tribolium			
anaphe	10	0·225 ± 0·008	0·188–0·257
audax	10	0·157 ± 0·003	0·145–0·171
brevicornis	10	0·388 ± 0·011	0·324–0·431
castaneum	10	0·203 ± 0·003	0·192–0·217
confusum	10	0·250 ± 0·008	0·204–0·300
destructor	10	0·174 ± 0·001	0·171–0·182
freemani	10	0·238 ± 0·003	0·222–0·262
madens	10	0·260 ± 0·011	0·190–0·326
Alphitobius			
diaperinus	5	0·278 ± 0·003	0·255–0·305

[a] Number of beetles of each species sampled.
[b] Twenty spermatids from each beetle were measured, but the standard error for each species is based on the variance of the 10 individual beetle means.

Table 2. *Results of the Student–Newman–Keuls (SNK) multirange test on the distribution of DNA values of individuals*

Species	Mean DNA values (pg)
T. audax	0·157[a]
T. destructor	0·174[a]
T. castaneum	0·203[b]
T. anaphe	0·225[c]
T. freemani	0·238[cd]
T. confusum	0·250[d]
T. madens	0·260[d]
T. brevicornis	0·388[e]

Mean DNA values of species with the same letter are not significantly different at $\alpha = 0{\cdot}05$.

The eight species of *Tribolium* and *Alphitobius diaperinus* gave the mean DNA values of spermatids reported in Table 1. As can be seen, the DNA content of *Alphitobius diaperinus* falls within the range of variation found among the species of *Tribolium*. These range from 0·157 pg in *T. audax* to 0·388 pg in *T. brevicornis*, that is over a twofold variation in genome size.

The SNK multirange test (Sokal & Rohlf, 1981) was also applied to test the significance of inter- and intraspecific differences. The eight species of *Tribolium* fall into five groups according to this test: (a) *T. audax* (0·157 pg) and *T. destructor* (0·174 pg), (b) *T. castaneum* (0·203 pg), (c) *T. anaphe* (0·225 pg) and *T. freemani* (0·238 pg), (d) again *T. freemani*, *T. confusum* (0·250 pg) and *T. madens* (0·260 pg), and (e) *T. brevicornis* (0·388 pg), as reported in Table 2.

The individual heterogeneity within each species was also measured by the same test. This produced

Table 3. *Results of a single classification ANOVA and SNK multirange test for heterogeneity of DNA values of individuals within each species*

Species	Mean squares[a]		F ratio	No. of significantly different groups
	Between beetles	Within beetles		
T. anaphe	1596·2	86·01	18·56***	2
T. audax	176·2	43·44	4·06***	3
T. brevicornis	2503·6	327·93	7·63***	3
T. castaneum	251·0	64·02	3·92***	2
T. confusum	1524·0	273·0	5·58***	4
T. destructor	50·2	26·1	1·92 n.s.	—
T. freemani	277·7	86·6	3·21**	2
T. madens	2777·8	90·33	30·75***	2

*** significant at $\alpha = 0{\cdot}001$, ** $\alpha = 0{\cdot}01$, n.s. = non-significant.
[a] M.S. values are multiplied by 10^{-3}.

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Table 4. *Nested analysis of variance*

Variance source	D.F.	M.S.[a]	*F*	Variance component
Between species	7	1003	137***	49·79
Between individuals within species	72	7·3	4·50***	2·85
Between spermatids within beetles	1520	1·63	—	16·29

*** significance at $\alpha = 0{\cdot}001$.
[a] M.S. values are multiplied by 100.

Table 5. *Average genome size differences (GSD) from pairwise comparisons between conspecific individuals and non-conspecific individuals of* Tribolium

Level	Mean genome difference ± S.E. (pg)	No. of pairwise comparisons
Individuals within species	0·0236 ± 0·0049	360
Species within the genus	0·0829 ± 0·0111	28

significant differences of F values in an analysis of variance for all species except *T. destructor*. From two to four groups of mean individual genome sizes could be distinguished in every species of *Tribolium* except for *T. destructor*, showing a remarkable degree of intraspecific variation (Table 3). A nested analysis of variance (Sokal & Rohlf, 1981) was applied to obtain the relative contributions of inter-, intraspecific differences, and within-individual variability to the whole variance for the total distribution of spermatid measurements (Table 4). The largest part of this variance (72·23 %) can be ascribed to the differences between species, whereas the differences between individuals within species accounted for only 4·13 %, and the differences between spermatids within individuals for 23·63 %. Nevertheless, either the inter-specific or intraspecific source of variance was highly significant as demonstrated by the F values.

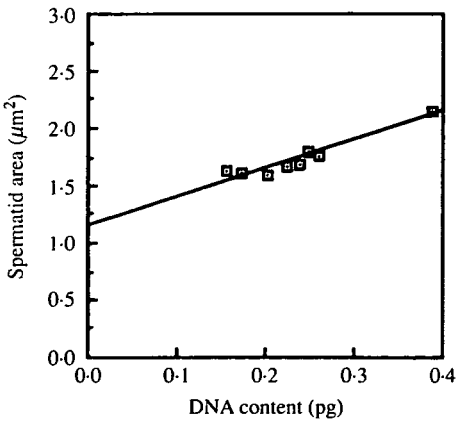

Fig. 1. Linear regression of DNA content *vs.* spermatid area in the eight species of *Tribolium*.

Gold & Amemiya (1987, p. 484) proposed two equations to measure genome size distances at each taxonomic level by calculating the average genome size difference between individuals drawn at random. The average genome size difference between two different *Tribolium* species is 0·083 pg and that between two conspecific individuals is 0·024 pg (Table 5). Therefore, two randomized individuals of the same species differ to about 30 % of the randomized difference between two non-conspecific individuals.

Linear regression of the mean DNA content against mean spermatid area per species (Fig. 1), was highly significant ($r = 0{\cdot}96$, $P < 0{\cdot}001$) but the mean DNA content does not provide a significant correlation with the mean body lengths of seven species of *Tribolium* given by Hinton (1948), and for *T. audax* by Sokoloff (1972), mainly due to the deviation caused by *T. destructor*.

4. Discussion

(i) *Interspecies variation*

There are two models of variation that can account for the evolutionary differences in genome size of closely related species. The first one is characterized by a discontinuous variation of quantum differences in genome size giving significant deviations in respect to normality as has been demonstrated in many groups of plants (Narayan, 1983; Sims & Price, 1985; Raina *et al.* 1986; Labani & Elkington, 1987). The second assumes small, continuous and overlapping

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changes of genome sizes across species, with no deviation from normality, as seen in several groups of animals (Bachmann *et al.* 1972, 1985; Bianchi *et al.* 1983; Gold & Amemiya, 1987; Ragland & Gold, 1989). However, Labani & Elkington (1987) pointed out that some of the discontinuous distributions of genome sizes may be derived from a non-random or incomplete sampling of species within a plant taxon.

Our results on eight species of *Tribolium* (Table 1) have been obtained by a large and uniform sampling either in the number of measured spermatids or in the screened individuals per species. This provides a solid base from which to evaluate the pattern of distribution of genome sizes both inter- and intraspecifically. The range of nuclear DNA content in *Tribolium* extends over a twofold difference from 0·157 pg of *T. audax* to 0·388 pg in *T. brevicornis* and this range includes the value found in *Alphitobius diaperinus* (0·278 pg) which should be taken as an outgroup species. The SNK multirange test used to discriminate among the mean individual values of DNA content gives five significantly different groups whose averaged gains or losses of genome size are 0·02–0·04 pg mostly. Only *T. castaneum* and *T. brevicornis* can be clearly distinguished from the other species while the remaining six display some overlapping of two or three mean values (Table 2). Therefore, the differences between species are mainly due to rather small shifts in the DNA amount, if we remove *T. brevicornis* from consideration.

The clear correlation between the mean genome sizes and mean spermatid area per species of *Tribolium*, $r = 0{\cdot}96$, $P < 0{\cdot}001$, is in agreement with those reported by other authors in many animals (Olmo, 1983; Szarski, 1976). This can be easily explained assuming that the different species of *Tribolium* display a similar degree of DNA condensation in their spermatids, the observed areas being a direct consequence of the DNA content. In contrast, the adult body lengths for the species of *Tribolium* gave a non-significant correlation with their mean DNA contents ($r = 0{\cdot}320$). Similar correlations have been found by Fox (1969) in beetles of the genus *Dermestes*, by Hinegardner (1973) in molluscs, and by McLaren *et al.* (1989) in copepod crustaceans, but not in most other animals (John & Miklos, 1988). Unexpectedly, the genome sizes of *T. castaneum*, *T. confusum* and *T. madens* also showed a highly significant correlation ($r = 0{\cdot}999$, $P < 0{\cdot}001$) with the shortest development period under optimal conditions as reported by Sokoloff (1972) for these three species.

The genomes sizes among species of *Tribolium* do not show a consistent interrelationship with either chromosome numbers, $2n = 20$ in *T. castaneum*, *T. madens*, *T. freemani* and *T. audax* and $2n = 18$ in the remaining species (Smith, 1952; Moore & Sokoloff, 1982), or genetic distances based on allozymic analysis (Wool, 1982). The only possible correspondence between genome sizes and current phylogenetic assumptions comes from the suggestion that the species with the highest genome size, *T. brevicornis*, is the ancestral species (Hinton, 1948; Sokoloff, 1978) and that evolution by decrease in genome size might be coupled to specialization as found in other organisms (Hinegardner, 1976; Cavalier-Smith, 1985).

(ii) *Intraspecies variation*

Although most authors (Mirsky & Ris, 1951; Rees & Jones, 1972; Sparrow *et al.* 1972; Bennett & Smith, 1976; Olmo, 1983; Cavalier-Smith, 1985) once assumed that nuclear DNA content is constant within a species or that any variation would be negligible, several recent papers have remarked the importance of intraspecies variation (Price *et al.* 1981; Sherwood & Patton, 1982; Bennett, 1985; Laurie & Bennett, 1985; Gold & Amemiya, 1987; Johnson *et al.* 1987; Ragland & Gold, 1989). Our findings support this, as significant differences in the mean DNA content between individuals were found in all but one of the eight species of *Tribolium* examined (Table 4). Some individuals, as for instance in *T. anaphe*, differ by as much as 25% in their genome sizes. This was an unexpected finding following the presumed high genetic homogeneity of laboratory strains like ours. The diversity of genome sizes between beetles of most species of *Tribolium* might come from different amounts of 'junk' or 'inert' DNA, but not of the informative DNA which would seem clearly unreasonable. Cytogenetic analyses to determine the amount of constitutive heterochromatin by C-banding or a molecular screening to measure that of highly repeated DNA among conspecific beetles may give an answer to the reported intraspecific variability of DNA content in *Tribolium*.

Another approach to the issue of intra-and interspecific variation of genome size in *Tribolium*, which provides additional support to the previous tests, comes from the nested analysis of variance. This statistical treatment allows us to divide the total variance in both levels of variation plus the error, issued from intraindividual variation. Both kinds of variation were statistically significant, but, as expected, the heterogeneity between species was strikingly higher than that within species (Table 5). Similar results were also obtained in North American sunfishes (Ragland & Gold, 1989); however, the mean differences in their genome sizes were smaller (6%) than those found within species of *Tribolium*.

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