

Amount of ribosomal DNA and seed protein content in the genus *Triticum*¹

F. Maggini, A. Blanco, D. Licci and M.J. Carmona

*Institute of Genetics and Institute of Plant Breeding, University of Bari, I-70126 Bari (Italy), and Institute of Cellular Biology, Madrid (Spain), 7 December 1979***Summary.** rRNA/DNA hybridization experiments were performed and seed protein content was determined in several lines of 4 species of *Triticum* (wheat). No correlation between rRNA gene number and seed protein content was found.

By the molecular hybridization of rRNA to total DNA it has been demonstrated that the ribosomal cistron number is not constant in different lines, strains or varieties of the following species: *Drosophila melanogaster*², *Ambystoma mexicanum*³, *Zea mays*^{4,5}, *Pisum sativum*⁶, *Triticum vulgare*^{7,8} and *Allium fistulosum*⁹. In most cases the range of rDNA variation was approximately 2-fold. In a preliminary report on 4 different lines of *Zea mays*, Phillips¹⁰ found no obvious relationship between rDNA amount and protein level of the seedlings. The aim of the present study was to determine possible differences in rDNA content among diploid and tetraploid species of *Triticum* and possible relationships between rDNA amount and seed protein content.

Materials and methods. Plants of several lines or cultivars of 2 diploid and 2 tetraploid species of *Triticum* (*T. boeoticum*) ($2n=2x=14$), *T. urartu* ($2n=2x=14$), *T. dicoccoides* ($2n=4x=28$) and *T. durum* ($2n=4x=28$) were grown in the greenhouse at room temperature. Young leaves were used for the DNA extraction, performed as previously described¹¹. For the labeled ribosomal RNA preparation 50 hypocotyls of *T. durum* cv. Cappelli were grown in White's solution containing 100 µg/ml of ³H-5,6-uridine (Amersham) under continuous agitation by magnetic stirring. After 72 h the material was 'chased' with unlabeled uridine at 200 µg/ml for 6 h. rRNA was extracted and purified as previously described¹¹. The specific activity was 58,000 cpm/µg. ³H-rRNA/DNA hybridization experiments were performed by the Gillespie and Spiegelman technique¹². The DNA-loaded filters were immersed for 12 h at 60 °C in

a 2×SSC solution containing a saturating amount of ³H-rRNA (2 µg/ml). After ribonuclease digestion, the washed and dried filters were counted in a mark I scintillation counter (Nuclear Chicago). The percent hybrid values are the mean of 4 determinations. Protein content analyses were carried out by means of an auto-analyzer, using 3 samples of 1 g of seed per line. The 5.7 factor was used to convert the nitrogen content into the crude protein percentage.

Results and discussion. The results of the hybridization experiments are reported in the table. As expected, an intraspecific variation of rDNA amount is present in the 4 species of *Triticum*. The extent of this variation ranges from 1.41–2-fold and this is in agreement with the previous reports in animal and plant species. According to Macgregor¹³, sister chromatid exchanges and unequal meiotic crossing-over might be partly responsible for such intraspecific variation of the ribosomal cistron number. Buongiorno-Nardelli et al.¹⁴ have suggested that variation in rDNA content may be introduced during DNA extraction. Our results from 7 replicate DNA preparations eliminate this possible source of error. Hence our data represent heritable differences in rDNA gene redundancy.

Our hybridization data were obtained using rRNA of *Triticum durum*; this is possible since competition experiments have shown that homology between rRNA from different species of the same genus is near 100%¹⁵. By the comparison of the per cent rDNA content in *Triticum* species we can see that appreciable differences between diploid and tetraploid species do not exist. This is in

rDNA and seed protein content among diploid and tetraploid species of *Triticum* (Mean ± SE)

Species	Line or cultivar*	rDNA (%)	Protein content (%)
<i>T. boeoticum</i> Boiss. ($2n=14$)	G 1882 A	0.075 ± 0.003	24.66 ± 0.56
	B	0.082 ± 0.004	
	G 2512	0.108 ± 0.003	27.37 ± 0.79
	G 2574 A	0.156 ± 0.005	
<i>T. urartu</i> Tum. ($2n=14$)	B	0.161 ± 0.004	25.63 ± 0.60
	G 1903 A	0.087 ± 0.002	
	B	0.084 ± 0.004	22.19 ± 0.77
	G 1545	0.095 ± 0.003	
	G 1546 A	0.123 ± 0.005	21.64 ± 0.31
	B	0.119 ± 0.004	
<i>T. dicoccoides</i> Körn. Schweinf. ($2n=28$)	G 3095	0.092 ± 0.003	22.02 ± 0.41
	G 3102	0.096 ± 0.005	24.31 ± 0.11
	G 2040	0.152 ± 0.004	21.55 ± 0.30
	Cappelli A	0.108 ± 0.003	15.49 ± 0.93
<i>T. durum</i> Desf. ($2n=28$)	B	0.110 ± 0.002	
	Creso A	0.132 ± 0.004	15.14 ± 0.23
	B	0.138 ± 0.005	
	Appulo A	0.070 ± 0.001	14.42 ± 0.78
	B	0.073 ± 0.002	
	Valgerardo	0.083 ± 0.003	16.04 ± 0.63
	Valnova	0.075 ± 0.003	16.29 ± 0.17
	Maristella	0.111 ± 0.004	14.98 ± 0.20
	Capeiti	0.124 ± 0.005	14.45 ± 0.20
	Ranger	0.143 ± 0.005	16.84 ± 0.13
	Valfiora	0.086 ± 0.003	17.37 ± 0.35
	Casteldelmonte	0.137 ± 0.004	14.71 ± 0.54

* A and B represent 2 duplicate DNA preparations of the same line or cultivar.

contrast to the results obtained by Siegel et al.¹⁶ in the genus *Nicotiana*, where diploid species possess about twice the rDNA percentage with respect to tetraploid species, so that the number of 80S rRNA cistrons is roughly the same in both. On the other hand, Siegel's data have not been confirmed by Cullis in *Nicotiana*¹⁷ and in *Datura*¹⁸ and by Maggini et al. in *Scilla autumnalis* and *Urginea maritima*¹⁹.

Many factors, including rRNA cistrons, influence the level of protein in the seed. We thought that the ribosomal cistron number might correlate with the total protein content of the seed. This is not the case in *Triticum* species. In fact in the diploid and tetraploid species studied there is no correlation between rDNA percentage and seed protein content. In this context Phillips¹⁰ hypothesized that in *Zea*

mays only a few rRNA genes are transcriptionally active, while the bulk of rRNA genes are useful only at certain developmental stages or under stress conditions. Since no differences in the DNA content per nucleus were found among 25 varieties of common wheat (*T. aestivum*) by Nishikawa and Furuta²⁰, we feel that an intraspecific stability of DNA amount per genome exists in each of the 4 *Triticum* species studied. As a striking example, assuming 24.2 pg as the DNA content per 2C nucleus in *T. durum*²¹ and 2×10^6 daltons as the molecular weight of the rRNA, we calculated rRNA gene numbers of 5500 and 10,400 respectively in *T. durum* cv. Valnova and Ranger, and the seed protein contents were 16.29% and 16.84% respectively. Phillips¹⁰ hypothesis could partly explain these results, but the question remains open.

- 1 Supported by CNR contract No. 74/0267.
- 2 F. Ritossa and G. Scala, *Genetics* 61, 305 (1969).
- 3 J.H. Sinclair, C.R. Carroll and R.R. Humphrey, *J. Cell Sci.* 15, 239 (1974).
- 4 S.A. Ramirez and J.H. Sinclair, *Genetics* 80, 495 (1975).
- 5 J.F. Givens and R.L. Phillips, *Chromosoma* 57, 103 (1976).
- 6 C.A. Cullis and D.R. Davies, *Genetics* 81, 485 (1975).
- 7 J. Mohan and R.B. Flavell, *Genetics* 76, 33 (1974).
- 8 R.B. Flavell and D.B. Smith, *Chromosoma* 47, 327 (1974).
- 9 J.H. Yampol and V.A. Pospelov, *Genetika* 14, 406 (1978).
- 10 R.L. Phillips, in: *Genetics and breeding of maize*. Ed. D.B. Walden. Wiley, New York 1977.
- 11 F. Maggini, P. Barsanti and T. Marazia, *Chromosoma* 66, 173 (1978).
- 12 D. Gillespie and S. Spiegelman, *J. molec. Biol.* 12, 829 (1965).
- 13 H.C. Macgregor, M. Vlad and L. Barnett, *Chromosoma* 59, 283 (1977).
- 14 M. Buongiorno-Nardelli, F. Amaldi and P.A. Lava-Sanchez, *Nature* 238, 134 (1972).
- 15 F. Maggini, R.I. De Dominicis and G. Salvi, *J. molec. Evol.* 8, 329 (1976).
- 16 A. Siegel, D. Lightfoot, O.G. Ward and S. Keener, *Science* 179, 682 (1973).
- 17 C.A. Cullis, *Chromosoma* 50, 435 (1975).
- 18 C.A. Cullis and D.R. Davies, *Chromosoma* 46, 23 (1974).
- 19 F. Maggini, P. Bassi and P. Stanziano, *G. Bot. ital.* 110, 331 (1976).
- 20 K. Nishikawa and Y. Furuta, *Jap. J. Breed.* 18, 30 (1967).
- 21 H. Rees and M.R. Walters, *Heredity* 20, 73 (1965).

The orientation of the golden hamster to its nest-site after the elimination of various sensory cues

Ariane S. Etienne¹

Laboratoire d'Ethologie, FPSE, Université II CH-1211 Genève 4 (Switzerland), 6 November 1979

Summary. Golden hamsters hoard food by carrying it back to their nest-site along a fairly direct path. 7 out of 12 animals continued to orientate in this way after passive transportation to the food source and the simultaneous elimination of visual, olfactory and acoustical cues. Experiments in which the hamsters tried to reach their nest-box from an unfamiliar place suggest that they orientate in a given direction with respect to a 'compass', the nature of which has still to be determined.

Preliminary observations and experiments have shown that within a familiar environment the golden hamster (*Mesocricetus auratus* Waterhouse) transports food to the habitual location of its nest-box by a fairly direct route, and that the animal maintains this orientation in spite of the elimination of various cues. The following 2 questions are therefore examined in this paper: a) Does the animal find the direct way back to its nest-site after being carried passively to the food source, and when visual, olfactory and acoustical cues have been simultaneously eliminated? b) Is the animal's direct orientation to the nest-site limited to the space it lives in, or can it 'home' from unfamiliar places? According to Bovey² this is the case for myomorph rodents which are released outside their home range; other authors, on the contrary, assume that there is as yet no convincing evidence for true navigation in rodents (see summary of literature in Joslin³).

All experiments took place under IR light in a cellar with thick external walls, double doors and no windows. The animals live under an artificial light dark cycle (dark: 18.00–06.00 h) and they are tested between 20.00 and 23.00 h, when the building is empty. Each individual occupies on its own a turnable arena ($\varnothing = 2.20$ m), where it

is introduced a few days before the beginning of the experiments and where it remains⁴ and moves freely throughout the experimental period. The arena's peripheral wall is made out of aluminium and is 50 cm high. 12 circular doors, hinged at the top, are set in the base of this wall at an angular distance of 30° from each other; all of them are permanently closed, except for one, through which the hamster can move freely in and out of a nest-box which is fixed to the outer side of the arena.

At the beginning of each trial, the animal is taken into a black box as soon as it leaves its nest-box; using a dim light, it is then deposited at the edge of a food bowl located in the centre of the arena, the orientation of the animal's head as well as the experimenter's own position being systematically varied from one trial to the next. When it has filled its pouches, the animal is allowed to go back to the arena's periphery, where it finds the nest entrance either directly or by searching movements along the circular enclosure.

The following procedures are used to eliminate various sensory cues simultaneously.

1) Optical cues. These cues are always eliminated, as the animals are filmed through an IR video-camera which is located above the centre of the arena and monitored from