Relative nuclear DNA amounts in various species and subspecies of the green toad group and in *Bufo bufo*

Species	Source	Relative DNA amount	SD	SE	Specimens
B. viridis turanensis	Frunze, Kirgizistan	211	19	8 (5)	1♀
B. viridis turanensis	Frunze, Kirgizistan	120	27	13 (4)	1♂
B. viridis arabicus	Haifa, Israel	105	10	4 (6)	2♂♂
B. viridis viridis	Izmir, Turkey	98	7	4 (3)	1♀
B. viridis ssp.	Corsica	103	7	3 (6)	2♂♀
B. viridis viridis	Germany	106	8	4 (4)	1♀
B. viridis boulengeri	Tafraoute, Morocco	109	6	3 (3)	2♂♀
B. viridis, all diploid data		107	14	3 (26)	
B. brongersmai	Tafraoute, Morocco	104	10	5 (4)	2♂♀
B. latastii	Afghanistan	145	14	6 (6)	3 juv.
B. calamita	Germany, Holland	100	5	2 (6)	2♂♀
B. bufo	Northern Spain	138	6	2 (6)	2♂♂

meter type GN-5. Series of specimens were prepared simultaneously, and all relative absorption values were converted to the same scale.

Result and discussion. The results of these measurements are compiled in the table. Except for 1 specimen of *B. viridis* from Frunze, Kirgizistan, all *B. viridis* have the same nuclear DNA content within the limits of experimental error. The relatively high average determination in the diploid specimen from Frunze seems to be due to the preparation, particularly since the value for the other specimen is very close to twice the average value for all *B. viridis*. This specimen undoubtedly is a tetraploid, even though there is no cytological confirmation. The existence of tetraploid 'populations' within diploid anuran species is a frequent occurrence and has also been documented for *Bufo*[7].

The relative DNA values of the table can be converted to approximate absolute values in pg of DNA by comparing them to published data for *B. viridis*, *B. calamita* and *B. bufo*[8,9]. These data have been repeatedly standardized against various species in our laboratory. Our calibration leads to higher values than those calculated by Olmo[10], which appear a bit too low. Best available estimates for the diploid nuclear DNA amounts are: *B. viridis* 11.8 pg, *B. calamita* 11.4 pg, *B. bufo* 14.8 pg (data sources in Olmo[10]). Therefore, to obtain diploid nuclear DNA amounts from the relative values of our table, the data should be divided by 9.0. Our data add 2 species of *Bufo* to the list of 20 for which specific nuclear DNA amounts have been published to date: *B. brongersmai*, 11.4 pg, and *B. latastii*, 16.0 pg. A value of about 11 pg seems to be typical for *Bufo*. The lowest recorded values are 8.9 pg for *B. koyanoiensis* (recalibrated after Olmo[10]) and *B. regularis*[9]. B. *regularis* is 1 of the African species having only 20 chromosomes instead of 22 in the diploid complement[11].

B. latastii, which was hitherto usually confused with *B. viridis*, has the highest nuclear DNA value recorded yet for a diploid *Bufo* species. This is the green toad species of the Central Asian mountain systems sharing a relatively low temperature preference and relatively large erythrocytes with *B. bufo*[4], which also has a high nuclear DNA amount. The tetraploid Frunze specimen of *B. viridis* (23.4 pg) has one of the highest anuran DNA amounts. Its erythrocytes are larger than those of diploid *B. viridis*, but on average smaller than those of large-sized *B. latastii* specimens[4].

Our data confirm the observation that the specific nuclear DNA content is a constant, and that closely related species may have similar or very divergent amounts. There is no evidence of a continuous variation, particularly no evidence for a gradual clinal change of genome size across the range. Such a change has been found by Miksche for several species of conifers[12], but there is no documented case of it for an animal species.

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Loss of redundant gene expression after polyploidization in plants

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Summary. Based on chromosomal location data of genes encoding 28 biochemical systems in allohexaploid wheat, *Triticum aestivum* L. (genomes AABBDD), it is concluded that the proportions of systems controlled by triplicate, duplicate, and single loci are 57%, 25%, and 18% respectively.

Ferris and Whitt[1] have recently presented compelling evidence of an extensive loss of duplicate gene expression after polyploidization in Catostomidae fish: 35%–50% of duplicate genes expressed in the most advanced tetraploid catostomids and 55%–65% in the most primitive species. They have also summarized the previous findings concerning this aspect of polyploid evolution in fish. We report here this type of calculation for allohexaploid wheat, *Triticum aestivum* L. (genomes AABBDD), a member of the well-known plant polyploid complex Aegilops-Triticum.

The loss of redundant gene expression in wheat was realized quite early. Riley[2] postulated the diploid-like status of some systems in tetraploid wheat on the basis of indirect evidence. Some of us[3,4] surveyed the distribution of genetic variants of 2 biochemical systems (genes for sterol esterification and the purothionins) in 22 species of the Aegilops-Triticum group and concluded that redundant genetic activity had been lost in 25% and 50% of the cases, respectively. All the observed losses seemed to occur in a non-random fashion, affecting the additional genomes and not the so-called pivotal ones.

The development by Sears[5,6] of the compensated nullitetrasomic series and other aneuploids of the *T. aestivum* cv. Chinese Spring has permitted several groups, including ours, to investigate the chromosomal location of genes that control different biochemical systems. These data, which are summarized in the table, permit not only the estimation of the percentage of gene triplication and duplication expressed, but also to discern where the pre-

sumed 'losses of redundancy', or inactivations, have taken place. Out of 28 sets of homoeologous systems, 16 (57%) are controlled by triplicate loci, 7 (25%) by duplicate loci, and 5 (18%) by single loci. In Catostomidae fish[1], the proportion of silenced loci per genome ranged from 18%, in the most primitive species, to 37%, in the most advanced ones. In hexaploid wheat, the proportions silenced in each genome are A (32%) > B (18%) > D (11%). The A and B genomes have coexisted in tetraploid wheat for some time before the more recent addition of the D genome to form the hexaploid, so the higher proportion of silenced loci in the A and B genomes, as compared with the D genome, could reflect the longer time they have been undergoing diploidization. However, the estimated figures for silenced loci in the wheat genomes must be considered quite high, as compared with those calculated for the catostomids, if the much longer evolutionary history of the latter (50 million versus 10,000 years) is taken into account. The number of triplicate genes expressed in wheat could be underestimated if those sets in which the 3 genes encode proteins with the same electrophoretic mobility were overlooked. However, this is unlikely, because such systems are also detected using aneuploids, on the basis of gene dosage responses, and their frequency seems to be low in an alloploid like wheat.

Chromosomal location of genes that control biochemical systems in allohexaploid wheat, *Triticum aestivum* (genomes AABBDD)

System*	Chromosomes	No. of loci	Ref.
Acp	4A, 4B, 4D	3 + 3	7
Adh	4A, 4B, 4D	3	8
α-Amy	6A, 6B, 6D	3	9
	7A, 7B, 7D		
Apep	6A, 6B, 6D	3	10
Epep	7A, 7B, 7D	3	11
Est	3A, 3B, 3D	3	12, 13
	6A, 6B, 6D	3	13
	7A, 7B, 7D	3	12
Got	3A, 3B, 3D	3	14
	6A, 6B, 6D	3 + 3	
Lpx	4A, 4B, 4D	3	11
	5A, 5B, 5D	3	
Pth	1A, 1B, 1D	3	15
β-Amy	4A, –, 4D	2	16
Px	–, 1B, 1D	2	13
Glut-sub	–, 1B, 1D	2	17
CM1, 2	–, 7B, 7D	2	18
NGE 16, 17	4A, –, 4D	2	19, 20
NGE 12, 13	4A, –, 4D	2	19, 20
NGE 5, 7	–, 3B, 3D	2	19, 20
Glut-sub	–, –, 4D	1	17
NGE 11	–, –, 7D	1	19, 20
NGE 2	–, 6B, –	1	19, 20
NGE 10	–, 6B, –	1	19, 20
NGE 14	–, 3B, –	1	19, 20

*Acid phosphatase (Acp); Alcohol dehydrogenase-NAD (Adh); α-Amylase (α-Amy); Aminopeptidase (Apep); Endopeptidase (Epep); Esterases (Est); Glutamate oxalacetate transaminase (Got); Lipoxidase (Lpx); Purothionins (Pth); β-Amylase (β-Amy); Peroxidase (Px); Glutenin subunits (Glut-sub); Chloroform:methanol proteins 1 and 2 (CM1,2); Non-gliadin 70% ethanol extracted proteins (NGE), homoeologies of NGE proteins are based on their characterization by aminoacid analysis, MW determination, solubility properties, etc. (*19, 20* and our unpublished results). Genes encoding gliadins, located in chromosomes of groups 1 and 6 (Wrigley and Shepherd[21]) have not been included in our calculations because in this system there are presumably tandem duplications.

One aspect of the data that might be significant, in the above context, is the fact that most of the presumably silenced loci are associated with endosperm proteins with no apparent enzymatic function, while most of the enzymatic loci are triplicated. This could indicate that some types of homoelogous sets are more constrained than others with respect to the loss of redundancy and that a greater fraction of the genomes would have to be investigated to get a reliable estimation. However, there are some arguments that could explain the high degree of gene silencing in wheat:

1. A more rapid gene loss could take place in the early history of the polyploid. As Ferris and Whitt[1] have pointed out, the process does not seem to be entirely at random. 2. An alloploid is in fact a 'permanent heterozygote' in which positive and negative heterotic interactions between homoeoalleles are effectively fixed. Negative heterosis can thus be considered as a driving force for the loss of duplicate gene expression. This effect would be initially more important in an alloploid (wheat) than in an autoploid (fish) for obvious reasons. 3. The disruption of the dosage balance between functionally related genes, implied in the loss of redundancy, has to be considered generally deleterious, and thus would tend to counteract whatever factors favor the loss. The disruption would be less drastic if the effective gene dosage is reduced by $^1/_3$ in a hexaploid (wheat) than by $^1/_2$ in a tetraploid (fish). Finally, available data on DNA content of hexaploid wheat and its ancestors[22], seem to indicate that, as in the case of fish[1], there is no apparent DNA loss matching the loss of redundant gene expression.

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