alone is less than 0.001. In contrast, the preference of the females remained unchanged over the 3 trials, so we can conclude that adult males of this selection line, but not females, can be conditioned to go towards the medium on which they were reared. Such sex differences in beetles on a learning task have been reported in another selection experiment involving running speed[9].

This result can be confirmed by altering the medium on which they were reared, so for G4, where the standard medium was used and where larger samples (150 of each sex) were available, the table shows that few males now preferred the garlic medium on the 1st trial and none on the 2nd. So few females went to the garlic medium on the 1st trial that there was an insufficient number for a 2nd trial.

These results can be contrasted with those obtained for *D. melanogaster*, where flies had to choose between a blank and a geraniol-baited tube. There, no evidence was obtained for conditioning (defined by an increased proportion going toward the normally aversive geraniol) at the larval or at the adult stage, but only for habituation (defined by little or no increase in preference predicted on successive trials). An explanation based on habituation is sufficient here to account for the G0 preference and the G3 and the G4 preferences of the females in the garlic line, however, conditioning may explain the performance of the G0 and the G3 and G4 males of the normal line. Thus, whether or not a preference indicates conditioning or habituation depends on genetic background and sex. Considering the biological restraints on learning in this situation[10], we might expect conditioning to occur more slowly in the Garlic line because of the aversiveness of the medium, and this tallies with the very rapid changes in preference between G3 and G4 when the medium was changed. It is not clear why the sex difference should occur, but it may involve the fact that the female uses the medium as an ovipositing site and not just as food. In view of the current interest in the genetics of learning in insects[11], and since a wide variety of antennal mutants of *Tribolium* are available, food-seeking and selection being mediated through these receptors[6], the present technique which combines selection with training may offer a convenient model for the study of learning.

9 I. M. Lerner and N. Inouye, in: Haldane and Modern Biology, p. 51. Ed. K. R. Dronamraju, John Hopkins, Baltimore 1968.
10 R. A. Hinde and J. Stevenson-Hinde, Eds., Constraints on Learning. Academic Press, London 1973.
11 W. G. Quinn and Y. Dudai, Nature *262*, 576 (1976).

Genome size in the green toad (*Bufo viridis*) group

K. Bachmann, A. Konrad, E. Oeldorf and H. Hemmer

Premedical Biology Program, University of Heidelberg, Im Neuenheimer Feld 504, D-6900 Heidelberg (Federal Republic of Germany); and Zoologisches Institut, University of Mainz, Saarstrasse 21, D-6500 Mainz (Federal Republic of Germany),
29 July 1977

Summary. Specimens of the green toad, *Bufo viridis*, from Morocco to Kirgizistan, have identical DNA amounts per erythrocyte nucleus. 1 specimen from Kirgizistan is a tetraploid. Of 3 closely related species, *B. calamita* and *B. brongersmai* have about the same DNA amount as *B. viridis*, while *B. latastii* from Afghanistan has 36% more DNA per nucleus.

The DNA content of the genome appears to be a constant for every plant and animal species, while it varies considerably even among closely related species wherever it has been studied. This variation is not random, but appears to be related to adaptive characters of morphology and physiology. From all indications, the mere amount of DNA in a genome is a decisive genetic determinant[1,2]. Since the variation within any species seems to be so much less than interspecific variation, changes in DNA amounts during speciation could be large and rapid ('saltatory changes'), and genome size within any species might be subject to regulatory influences. There is very little information on the evolutionary dynamics of the change in DNA content, and very few species have been investigated throughout their geographical range for DNA amounts. Therefore we wish to present a set of measurements on the nuclear DNA content of green toads covering nearly the whole distributional area of this widespread species. These measurements show that, within the accuracy of determination (10%), *Bufo viridis* has the same nuclear DNA content throughout. 1 of 2 specimens from Kirgizistan has twice this amount in erythrocyte nuclei. 2 related species, *B. calamita* and *B. brongersmai* from Morocco[3], where it is sympatric with *B. viridis*, have essentially the same DNA amount, while *B. latastii* from Afghanistan[4] has 36% more DNA in its erythrocytes.

Material and methods. Blood was obtained from living specimens[5] by puncturing the angular vein of the mouth with a micropipette. Blood smears were fixed in 10% formalin, then air-dried and stored dry for up to a few days before staining. Feulgen staining was preceded by various methods of DNA hydrolysis in order to exclude systematic errors stemming from the preparation[6]. Dye content of Feulgen-stained nuclei was determined at 558 nm using a Barr and Stroud Integrating Microdensito-

1 K. Bachmann, O. B. Goin and C. J. Goin, Brookhaven Symp. Biol. *23*, 419 (1972).
2 H. J. Price, Bot. Rev. *42*, 27 (1976).
3 M. S. Hoogmoed, Zoöl. Meded., Leiden *47*, 49 (1972).
4 H. Hemmer, J. F. Schmidtler and W. Böhme, Zool. Abh. Dresden *34*, in press (1978).
5 Sources of the animals are: *B. viridis*, Frunze, Kirgizistan, coll. Hemmer; Haifa, Israel, commercial dealer; Izmir, Turkey, coll. Prof. Dr Özeti, Izmir; Corsica, coll. Dr Back, Bonn; Germany, coll. Hemmer; Tafraoute, Morocco, coll. Schulte, Neuhaus; *B. brongersmai*, Tafraoute, Morocco, coll. Schulte, Neuhaus; *B. latastii*, Afghanistan, coll. Scheffler, Mühlheim; *B. calamita*, Germany, coll. Hemmer; Holland, coll. Dr Hoogmoed, Leiden; *B. bufo*, Northern Spain, coll. Gutsche, Aachen.
6 K. Bachmann and J. P. Bogart, Cytogenet. Cell Genet. *15*, 186 (1975).

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.

7 J. P. Bogart, Science *193*, 334 (1976).
8 F. H. Ullerich, Chromosoma *18*, 316 (1966).
9 K. Bachmann, Chromosoma *29*, 365 (1970).
10 E. Olmo, Caryologia *26*, 43 (1973).
11 J. P. Bogart, Evolution *22*, 42 (1968).
12 J. P. Miksche, Chromosoma *32*, 343 (1971).

Loss of redundant gene expression after polyploidization in plants

F. García-Olmedo, P. Carbonero, C. Aragoncillo and G. Salcedo

Departamento de Bioquímica, E. T. S. Ingenieros Agrónomos, Madrid-3 (Spain), 1 August 1977

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-