Hylid Frogs: Polyploid Classes of DNA in Liver Nuclei

Abstract. *Microspectrophotometric DNA determinations on liver nuclei of hylid frogs have revealed the presence of several polyploid classes of DNA in several specimens belonging to five species. All such specimens were breeding.*

The spontaneous occurrence of polyploid nuclei in liver cells is well known in rodents, and also occurs in several other vertebrate groups. In Amphibia the usual low incidence of nuclei in the liver with amounts of DNA corresponding to tetraploidy (*1*, *2*) can be accounted for by premitotic DNA doubling. During a comparative survey of DNA levels in amphibians, we have recently found amounts of DNA indicative of true polyploidy or polyteny in several species of tree frogs.

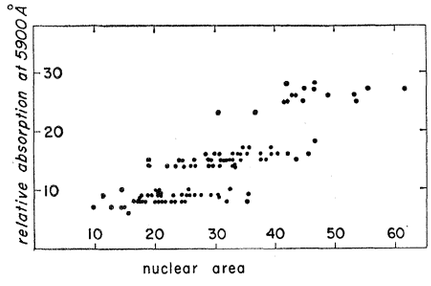

Fig. 1. Female *Pseudacris ornata*: Feulgen absorption versus nuclear area.

Nuclei isolated from liver tissue by a reported method (*2*) were stained for DNA by the Feulgen reaction after hydrolysis in 0.1*N* HCl at 60°C for various periods. The total dye content of single nuclei was determined by microdensitometry (*3*).

Usually all DNA determinations from one liver are distributed around a single value which is proportional to the amount of DNA present in the nuclei (*4*). Occasionally a few nuclei show twice the normal amount (Table 1, items 2, 5, 8, 17, 20), indicating a low level of premitotic DNA synthesis in otherwise diploid livers. In some instances, however, several distinct nonoverlapping absorption classes were found (Table 1, items 1, 6, 16, 18, 19, 21). Within each class the absorption was independent of nuclear area, but the average nuclear sizes associated with the different classes increased with the absorption levels (Fig. 1). This pattern indicates the presence of several ploidy classes (*5*), corresponding to the presence of multiple chromosome sets (true polyploidy) or multistranded chromosomes (polyteny) in these nuclei.

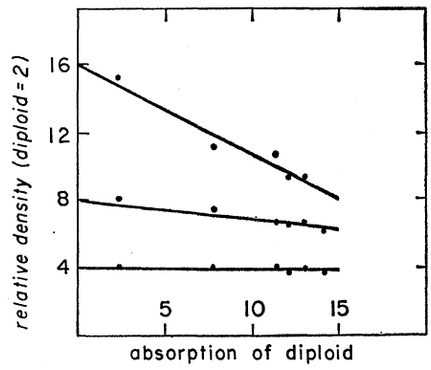

Fig. 2. Female *Pseudacris triseriata*: Feulgen densities of higher ploidy groups compared with diploid absorption values. Diploid absorption values (arbitrary scale units) have been changed by variation in duration of hydrolysis.

The high Feulgen absorption values obtained after optimal hydrolysis times of about 6 minutes result in density-dependent optical errors that preclude determination of the ploidy levels present by direct comparison of the absorption values, even when the measurements are performed at wavelengths off the absorption peak (Fig. 1). If the nuclei are stained after various hydrolysis times, the overall density of the stain can be varied; with decreasing overall density, the optical error decreases and the different absorption classes separate more markedly (Fig. 2). This effect can be used to determine the absorption ratios, in the absence of optical error, by extrapolating the ratios to zero density. In this way the ploidy values in *Pseudacris triseriata* have been determined as $2n$:$4.2n$:$8.9n$:$17.3n$. In the same species, average nuclear volumes associated with the four absorption classes relate to each other as 2.0:4.1:7.6:17.4. Neither of these series is significantly different from 2:4:8:16, the most probable levels of ploidy.

Determinations of the ploidy levels by other methods and elucidation of the chromosome pattern of these nuclei have not yet been possible; the mitotic index is low, chromocenters are not clearly defined in number, and all cells have one single nucleolus only, which can be extremely large in size in nuclei belonging to the higher classes of DNA.

We have found polyploid nuclei only in tree frogs of the family Hylidae, but in specimens of various genera—in five of the 11 hylid species examined.

Table 1. Summary of DNA measurements in hylid frogs. Numbers of measurements appear in parentheses.

Item	Species	Sex, age	Polyploid nuclei (%)			
			$2n$	$4n$	$8n$	$16n$
1 (100)	*Acris gryllus*	Early-breeding male	87	13	0	0
2 (50)	*Hyla brunnea*	Adult	98	2	0	0
3 (50)	*Hyla cinerea*	Adult male	100	0	0	0
4 (50)	*Hyla cinerea*	Adult female	100	0	0	0
5 (50)	*Hyla cinerea*	Adult female	96	4	0	0
6 (100)	*Hyla crucifer*	Breeding female	64	32	4	0
7 (50)	*Hyla crucifer*	Breeding male	100	0	0	0
8 (50)	*Hyla crucifer*	Young male	98	2	0	0
9 (50)	*Hyla crucifer*	Breeding male	100	0	0	0
10 (50)	*Hyla crucifer*	Breeding male	100	0	0	0
11 (50)	*Hyla femoralis*	Young	100	0	0	0
12 (50)	*Hyla gratiosa*	Young female	100	0	0	0
13 (50)	*Hyla ocularis*	Adult	100	0	0	0
14 (50)	*Hyla ocularis*	Adult male	100	0	0	0
15 (50)	*Hyla squirella*	Adult	100	0	0	0
16 (100)	*Pseudacris nigrita*	Breeding female	76	24	0	0
17 (50)	*Pseudacris nigrita*	Young male	96	4	0	0
18 (100)	*Pseudacris ornata*	Breeding female	44	40	16	0
19 (388)	*Pseudacris ornata*	Breeding male	60	33	7	0
20 (50)	*Pseudacris triseriata*	Adult male	98	2	0	0
21 (300)	*Pseudacris triseriata*	Breeding female	44	29	24	3

Such nuclei seem to be limited to breeding frogs—females with ripe ovaries and calling males taken at the breeding sites. Polyploidy seems to be limited to breeding females in *Hyla crucifer*. The six species that did not show polyploidy were all taken outside the breeding season.

This very high incidence of nuclei with amounts of DNA corresponding to higher polyploid classes, and the possible correlation with the reproductive cycle, should make these species interesting subjects for cytological and physiological studies.

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References and Notes

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Homoreactant: A Naturally Occurring Autoantibody in Rabbits

Abstract. *Homoreactant, a factor found in the serum of every normal rabbit tested thus far, reacts specifically with buried antigenic determinants on the Fab-fragment of papain-digested autologous and homologous γG-globulin. It appears that rabbits normally produce naturally occurring autoantibodies.*

The products of enzymatic digestions and controlled reductions and alkylations have proved exceptionally useful for describing the chemical and biological nature of the γG-globulin molecule. Of particular importance was the finding that papain digestion of rabbit antibody results in the formation of two antigenically and biologically distinct fragments, Fab and Fc. The crystallizable Fc-fragment has most of the biological properties of the intact antibody molecule; but the Fab-fragment has the antibody-combining site and, although univalent, retains its capacity to react with specific antigens (*1*).

Erythrocytes which are sensitized with immune Fab-fragments resemble erythrocytes sensitized with the incomplete antibodies to Rh (+) erythrocytes in that they do not agglutinate in saline, but they can be agglutinated by a suitable antibody to γG-globulin (*2*). Subsequently we observed that human O Rh (+) erythrocytes sensitized with Fab-fragment from immune rabbits could be agglutinated by a rabbit antiserum to human γG-globulin. Initially we believed that this was due to a cross-reacting antigenic determinant common to human and rabbit γG-globulin. However, we later found this peculiar antiglobulin factor in pooled or individual normal rabbit serum and attributed the activity to a heat-stable, nondialyzable, mercaptoethanol-resistant, γG-globulin factor (*3*). Because this factor differs from other antibodies (such as those called "anti-antibody," antiglobulin reagent, rheumatoid factor-like antibody, and antiallotype antibody), we initially assigned to it the term "homoreactant" to designate a normal γG-globulin that reacts with a fragment (Fab) derived by enzyme digestions of the γG-globulin of the same species (*3*).

We now report results obtained when the homoreactant of individual rabbit serum was tested against the Fab-fragments of γG-globulin isolated from the same rabbit. Our results in Tables 1 and 2 show, by agglutination and inhibition of agglutination, that these individual serums react with autologous as well as homologous Fab-fragments. From these findings we conclude that homoreactant has certain characteristics of an autoantibody whose specificity is directed toward buried autoantigenic determinants unique to the Fab-fragment.

Rabbit antibody to human erythrocyte (HEA) was produced in several rabbits by intravenous injections of washed, human O Rh (+) erythrocytes. The Fab-fragments of antibody to HEA were obtained after papain digestion of γG-globulins isolated from a pool of bleedings from several immune rabbits or a pool of bleedings from each of three individual immune rabbits (A9, A11, and A12). Serums were also collected from these individual rabbits prior to immunization. For the inhibition experiment the Fab-fragments were obtained from papain digests of γG-globulin isolated from several individual bleedings of six normal rabbits (B4, H2, H3, G3, G4, and G5). Precipitations with sodium sulfate and diethylaminoethyl cellulose (DEAE) chromatography were used to purify the γG-globulin from normal and immune rabbit serums (*4*). Papain digestions were performed according to the method of Porter (*5*), and the Fab-fragments were purified as described by Mandy, Stambaugh, and Nisonoff (*6*). No agglutination occurred when dilutions of Fab-fragments from antibody to HEA were mixed with O Rh (+) erythrocytes in saline. Sensitization of the erythrocytes by the Fab-fragments, however, was demonstrated by agglutination reactions with goat antibody prepared against rabbit γG-globulin.

Table 1. Agglutination by normal serums of erythrocytes sensitized with the homologous and autologous Fab-fragment of antibody to HEA. All serums were heat-inactivated and absorbed for heteroagglutinins.

Serums before immunization	Reciprocal dilutions of serums				
	16	64	256	1024	Saline
A9, Fab from antibody to HEA					
A9	4+	2+	1+	0	0
A11	3+	1+	0	0	0
A12	4+	3+	1+	0	0
A11, Fab from antibody to HEA					
A9	4+	3+	1+	0	0
A11	3+	1+	1+	0	0
A12	4+	3+	1+	0	0
A12, Fab from antibody to HEA					
A9	4+	3+	1+	0	0
A11	4+	1+	1+	0	0
A12	4+	4+	2+	0	0

Equal volumes of a 2-percent suspension of washed human O Rh (+) erythrocytes and solutions of Fab (0.31 mg/ml) from antibody to HEA were mixed and incubated at 37°C for 1 hour. The sensitized cells were then washed three times with saline and reconstituted in saline to a 2-percent suspension.

Agglutination reactions were performed by mixing equal volumes (0.1 ml) of each of a serial fourfold dilution of serum and of sensitized erythrocytes. After incubation at room temperature for 15 minutes, the mixtures were centrifuged in a Serofuge (Clay-Adams), and each was scored for agglutination between 0 and 4+.

Inhibition of agglutination was carried out by incubation of equal volumes of various dilutions of the inhibitor with a standard 1:8 dilution of normal serum for 1 hour at 37°C. Sensitized cells were then added, in-