proportional to the diameter of the ring of immune precipitate formed, and is estimated by reference to a standard curve relating ring diameters to known concentrations of the purified globulin. The antiserums were prepared by immunization of rabbits with purified antigens isolated from normal human serum. The antiserums were made specific by absorption with the slow fragment of a papain digest of fraction II gamma globulin. The preparation of the purified antigens for standardization and of the antiserums has been previously described (*3*). The values obtained for normal serum per 100 ml (γ_2, 1335 mg S.D. 267; γ_{1A}, 178 mg S.D. 60; and γ_{1M}, 103 mg S.D. 22) are slightly higher than those reported by Heremans (*4*).

The results of the quantitative analysis of the gamma globulins in various fluids are shown in Table 1. Three types of fluid were found. In one, represented by parotid saliva, colostrum, and lacrimal secretions, there is little or no γ_2-globulin and the gamma globulin is predominantly γ_{1A}. In a second type represented by small intestinal fluid and bile, γ_2-globulin is the predominant gamma globulin, but relatively large amounts of γ_{1A} are present and the ratio γ_2/γ_{1A} is significantly lower than that of serum. Similar results have been found with human bronchial secretions (*5*). In a third type, represented by vaginal and prostatic secretions, the ratio of γ_2/γ_{1A} did not differ significantly from that of serum. However, both of these fluids showed variation in the proportions of the gamma globulins. Moreover, as a result of very low protein content in several vaginal fluids, neither γ_2 nor γ_{1A}-globulin was detected, thus precluding determination of a mean γ_2/γ_{1A} ratio. Although studies on more samples are necessary, the results suggest that amniotic fluid represents a fourth type of fluid with a high γ_2/γ_{1A} ratio compared with serum.

Table 1. Gamma globulins and mean γ_2/γ_{1A} ratios in normal human serum and various fluids. The number of samples for each fluid is given in parentheses.

Mean of globulin fraction (mg/100 ml)		Mean γ_2/γ_{1A}	P*
γ_2	γ_{1A}		
	Serum (14)		
1335	178	8	
	Parotid fluid (12)		
0†	28	> 1	
	Colostrum (5)		
0†	151	> 1	
	Lacrinal fluid (8)		
0†	7	> 1	
	Intestinal (small) fluid‡ (6)		
153	74	3.8	.01
	Bile ‡ (8)		
143	53	2.6	.025
	Prostatic fluid (17)		
157	26	10	.5
	Vaginal fluid (16)		
37	6.3		.7
	Amniotic fluid (4)		
21	1.6	15	
	Cerebrospinal (4)		
7.9	1.8	5	

* P reported for comparison of γ_2/γ_{1A} ratios of serum and fluids by t-test (intestinal fluid) and analysis of median test with the chi-square method (bile, prostatic, and vaginal fluids). † The γ_2-globulin was detected in some of these fluids by the double diffusion precipitin test (Ouchterlony) but the amounts were too small for quantitative measurement. ‡ Values for the concentration of γ_2- and γ_{1A}-globulins of these fluids are on concentrated samples.

Four cerebrospinal fluids from patients with cerebrovascular disease contained slightly higher ratios (mean γ_2/γ_{1A} ratio 5/1) than normal serum. The γ_2/γ_{1A} ratio of cerebrospinal fluids obtained from two patients with disseminated sclerosis was 176/1 indicating that the increase in total cerebrospinal fluid gamma globulin in these two cases is almost entirely the result of an increase in γ_2-globulin.

The γ_{1M}-globulin was detected in trace amounts in many fluids but was present in measurable quantities only in colostrum. This indicates that the relative γ_{1M} concentration of the fluids (excepting colostrum) is not greater, and probably lower, than that of serum.

The data suggest that the gamma globulins of these fluids are not derived from serum by simple transudation. This is indicated not only by the relatively high γ_{1A} content of these fluids, but by the low to absent γ_2-globulin, particularly in proportion to its high concentration in serum. However, changes in the original gamma globulin content of the fluids may have resulted from a differential susceptibility of the various types of gamma globulin to the proteolytic enzymes in these fluids. After the incubation of γ_2-globulin with concentrated parotid saliva no evidence of proteolysis could be detected. Further work is necessary, however, to exclude this possibility in other secretions such as bile and intestinal fluid.

The observation of Brambell *et al.* (*6*) that fraction III of papain-digested rabbit gamma globulin passes the placental barrier much more readily than fractions I and II suggests the presence of an "active transport site" on the protein molecule and supports the concept that gamma globulins are selectively transported from serum to certain secretions. The occurrence of a higher specific antibody titer in colostrum, feces, and vaginal mucous than in serum (*7*) also suggests that active transport is involved in the secretion of gamma globulins into these fluids.

During acute infections at mucous surfaces the antibodies in the fluid bathing the infected area are derived largely from lymph nodes in the immediate vicinity (*7*). The gamma globulins in these fluids may therefore reflect gamma globulin production of adjacent lymph nodes rather than serum gamma globulins.

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Color Polymorphism in Pacific Tree Frogs

Abstract. *Crosses between green and non-green* Hyla regilla *suggest that green color is determined by genes at two loci; each loci must have a dominant gene. Red is the result of a recessive gene and brown a dominant gene. The frequency of frogs with each color varies in different populations.*

The background colors of *Hyla regilla* may be classified into four main groups: red, green, grey, and brown. An individual frog may have one or more of these colors. When more than one color is present, the colors are discrete. The animal may get very pale or very dark; the colors maintain their integrity. The intensity of the color varies seasonally and, to some extent, with age. The frequencies of the colors vary geographically and from year to year at a single locality. In the experi-

Table 1. The total number of Pacific tree frogs collected during the years 1961 to 1963, and the frequency of frogs of each color among those obtained from each area.

Locality	Total number	Frequency of occurrence (%)			
		Green	Brown	Grey	Red
Northwest coastal areas	51	88	90	2	33
Willamette-Puget valleys	178	73	68	3	8
Northwest mountains	439	7	83	25	9
North and central California lowlands	177	46	56	29	8
Sierra Nevadas	216	5	28	42	9
Southern and Baja California mountains	254	33	75	8	6
Southern and Baja California deserts	128	28	85	3	9
Southern and Baja California coastal areas	164	8	94		15
Subtropical Baja California	150	20	78	13	14

ments reported here, pairs of Pacific tree frogs were obtained from their natural habitat and from the laboratory stock. Pairs producing fertile eggs were measured and their color and head and body patterns were recorded in order to determine the nature of the inheritance of the colors.

When collected from their natural habitat, pairs of frogs in amplexus usually produced eggs, the majority of which were fertile. About 30 percent of those paired in the laboratory produced eggs; of these approximately 50 percent were fertile. Each fertile cross resulted in 200 to 1000 eggs. Thirty to 150 eggs were placed in Pyrex dishes; tadpoles were fed Purina Rabbit Chow (EW-5320). Froglets were fed *Drosophila* and house flies. From 1 to 73 frogs metamorphosed from each cross; no crosses were lost.

The baby frogs were marked by toe-clipping, and notes on their colors were made at transformation and at monthly intervals thereafter to determine whether or not the colors present changed as the animals matured.

Histological examination of the dorsal skin revealed that red and green frogs possess large melanophores and crystalline guanophores. Above the melanophore-guanophore layer in red frogs is a layer of cells (allophores) containing a red pigment insoluble in water, alcohol, or petroleum ether. Xanthophores occupy the same position in green frogs. The yellow pigment of the xanthophore has an absorption spectrum characteristic of the carotenoids. Carotenes and xanthophylls (the two main subgroups of the carotenoids) were separated by partition between two solvents, followed by column chromatography. The carotene fraction is largely beta-carotene, while the majority of the xanthophyll fraction contains four to five xanthophylls which are almost completely esterified.

The offspring of all crosses between non-red males and non-red females that produced at least one red offspring were examined. From 38 such crosses, 189 non-red and 91 red frogs were observed. Since the crosses were selected by the identification of at least one red offspring (that is, all crosses in the study were not used) it is necessary to subtract one red frog from each cross (proband correction) before statistical analysis. The corrected results of 189 non-red and 53 red frogs yielded a Chi-square (χ^2) value of 1.219 ($p > .99$) when tested against the hypothesis that red color is the result of the homozygous condition of an autosomal recessive gene *rr*.

Until 1963, the offspring of crosses between red parents were not available. Preliminary transformations from three of this year's crosses indicate agreement with the hypothesis. I. Red males × red females produced 8 offspring, all red. II. Red males × non-red females produced 3 red and 2 non-red offspring [theoretically (1 : 1), $\chi^2 = 0.200$]. III. Non-red males × non-red females produced 22 non-red and 6 red offspring [theoretically (3 : 1) $\chi^2 = 0.191$].

Analysis of the inheritance of green in tree frogs was accomplished by examination of crosses of (i) green × green; (ii) non-green × non-green; (iii) green × non-green. Preliminary examination of our crosses suggests that the green color is the result of two pairs of factors; the dominant factor of each pair is necessary for the production of the green color. The homozygous recessive condition of either (or both) pairs produces a non-green frog.

(i) Green × green. Five crosses of this type produced non-green as well as green frogs. On the basis of our hypothesis the parental genotypes should be: (1) *OoGg*; (2) *OOGg*; (3) *OoGG*; or (4) *OOGG*. A ratio of 9 green to 7 non-green offspring would be expected from a cross of (1) × (1), while a 3 : 1 ratio would be predicted from crosses between any combinations of the first three genotypes. The fourth genotype would be expected to yield all green offspring regardless of the genotype of the other parent.

Each cross was tested (χ^2) for both ratios. Four of the five crosses were of the 9 : 7 ratio (a total of 81 green and 68 non-green, $\chi^2 = 0.070$; $p > .99$), while the fifth cross was of the 3 : 1 ratio (7 green and 1 non-green, $\chi^2 = 0.670$; $p > .99$). A proband adjustment was not required in this instance as the crosses were selected on the basis of the color of the parents alone.

(ii) Non-green × non-green. Examination of the inheritance of green color shows that crosses between non-green frogs have the following genetic makeup: (1) *ooGG* × *Oogg*; (2) *ooGg* × *OOgg*; or (3) *ooGg* × *Oogg*, and may yield a theoretical 1 : 1 ratio (Nos. 1 and 2) or a 1 : 3 ratio (No. 3). Each of 25 crosses of non-green with non-green frogs was individually tested against both expected ratios, and the results pooled. Thirteen crosses were separately found to fit a 1 : 1 ratio. These crosses were then pooled and a χ^2 analysis of the 47 green and 43 non-green frogs was made ($\chi^2 = 0.057$, $p > .99$).

(iii) Green × non-green. Crosses of this type could lead to four theoretical offspring ratios, depending upon the parental genotypes. These genotypes and their combinations are numerous and cumbersome and are not listed. The theoretical offspring ratios of green to non-green are 3 : 1, 1 : 1, 1 : 3, 3 : 5. The 12 crosses between green and non-green in our laboratory all fit at least one of these ratios (χ^2, $p > .95$). The offspring of these crosses (green with non-green) were as follows: 6 : 4; 6 : 2; 5 : 3; 6 : 1; 6 : 3; 4 : 1; 8 : 3; 5 : 3, 5 : 5; 2 : 4; 1 : 4; 2 : 5.

Albino *Hyla regilla* have been found in nature (*1*), and A. Hight, at the University of California, Santa Barbara, has raised 14 albinos to the size of sexually mature adults. 'Partial' albinos have patterns on the head and back, and lack background melanin: these characteristics may be the result of gene blockage further along in the metabolic pathway than in total albinism.

Some albinos have allophores which

impart a clear red color to the vertebral stripe. Albinos with xanthophores appear green when placed on a dark background in reflected light. Other albinos with neither allophores nor xanthophores may be 'brown' albinos. The guanophores are not continuous across the back of the frog. A narrow band around each dorsal stripe where the chromatophores are absent accounts for the conspicuous light area surrounding the head and body patterns.

Live tree frogs were collected from more than 100 localities from British Columbia to Baja California during the years 1961 to 1963. The colors of the frogs from some of these localities are listed in Table 1. The frequency of red frogs is greatest in xeric areas; the hue and intensity of the red color ranges from a bronze in the frogs from Lake Arrowhead and Oregon to a more 'iron-rust' red in frogs from San Diego and Baja California.

Pyburn (*2*) found that the red color of the vertebral stripe of the cricket frog (*Acris crepitans*) is controlled by a single dominant gene. Other crosses led Pyburn to conclude that green is also under the control of a dominant allele of a single gene. Our data suggest that green color in *Hyla regilla* is determined by genes from at least two different loci. After the breeding season, green cricket frogs "fade" to grey and in the spring become green once more. Pacific tree frogs lighten and darken in response to hormones, light, and temperature, but the colors retain their structural integrity. A different structural and metabolic basis for the colors in these two genera is suggested.

Green frogs vary in hue and intensity from a dark, almost black, green (Sierra Mountains) to grass-green individuals (Southern California and Baja California) to blue frogs (Southern Oregon). The blue animals were collected (along with animals with red, brown, and green colors) in 1954 and again in 1961 in the same pond, which suggests that this blue is under genetic control. The blue color appears to be due to the Tyndall effect in the absence of xanthophores. The brown frogs range in color from yellow-tan to almost black. Preliminary examination of the 1962 crosses suggests that brown is controlled by at least one dominant gene; the variability suggests a much more complex system and more work is required.

Close examination of the frogs thus reveals that there is great variability within each color phase. The large number of different shades of green could be partially explained on the basis of the different alleles at the two loci involved in determining green color—that is, different alleles controlling the amount of guanine or carotenoid pigment produced. There may also be an interaction of one or a group of modifying genes, as was suggested by Volpe and Dasgupta (*3*) in the Burnsi complex in *Rana pipiens*.

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Rationale for a Universal Genetic Code

Abstract. *A mutation in the genetic code would place new amino acids in certain loci and entirely eliminate amino acids from other loci of practically all proteins in an organism. It is reasonable to postulate that mutations of this kind cannot supplant the original code. The genetic code, once established, would therefore remain invariant.*

During the past decade a considerable body of evidence has accumulated in support of the hypothesis that a sequence of the nucleotide triplets in the DNA of an organism bears a one-to-one correspondence to the sequence of amino acids in the proteins synthesized by that organism. This implies that, given the nucleotide sequence in a strand of DNA, the amino acid sequence in the protein corresponding to that strand could be predicted. The abstract set of rules which associates a nucleotide triplet with a given amino acid is known as the genetic code. The genetic code is expressed in the organism as a set of molecular interactions. The molecules taking part in these interactions are believed to be DNA, messenger RNA (mRNA), a low molecular weight amino acid adaptor RNA (aRNA, also called acceptor, transfer, or soluble RNA), and an amino acid activating enzyme (aa-enzyme).

The following relationship is now believed to exist among these molecular species (*1*): The mRNA is synthesized on DNA and is complementary to it, so that, in principle, if the sequence of mRNA nucleotides is known, the sequence of nucleotides in the DNA can be deduced, and vice versa. In the process of protein synthesis the different amino acids are ordered in the protein molecule according to the sequence of mRNA nucleotide triplets. Each triplet will be called a codon, a term coined by Crick. Each amino acid is matched to its codon by amino acid–specific aRNAs. The structure of aRNA is still incompletely understood but it can be visualized as a short double-stranded molecule that can carry an activated amino acid with a stereospecific configuration of nucleotides at one end which we will designate by the letter C (*2*) and which will only form a bond with one particular codon. There should be as many different adaptor molecules in an organism as there are different codons. The amino acid is attached to the adaptor molecule by an amino acid activating enzyme (*1*). This enzyme should have two sites, A and R. The amino acid attaches to site A while site E of a particular adaptor RNA molecule attaches to R (Fig. 1). Sites A and R, and E and C are essentially independent (*3*).

It is safe to say that the synthesis of both the aa-enzyme and the aRNA molecules are under genetic control. For the aa-enzyme this assumption can be justified because there is no evidence, nor known biochemical mechanism, for the self-replication of protein molecules. Independent self-replication of adaptor RNA can be ruled out on theoretical grounds: If these molecules were independent, then as errors in replication occurred a heterogeneous population of adaptor molecules would result which would completely destroy the precision of the protein synthesis mechanism, a precision known to exist on the basis of analysis of protein structure.

Since DNA can mutate, the structures of the aa-enzyme and of the aRNA are mutable. Since the operation