estrus[7], elevates the plasmatic level of gonadotropins[8], and increases the production of progesterone by the ovary[9]. Otherwise, after 3 weeks, destruction of the amygdala brings about a decline in the levels of FSH-RF[10] and elevation of those of LH-RF[11]. These activities seem to be mediated by the stria terminalis[8] which connects the amygdala and hypothalamus. On the contrary, stimulation of the hippocampus impedes ovulation in cyclic rats[12].

The limbic system has cyclic modifications in its oxidative metabolism which disappear with ovariectomy[2], experimental diabetes[13], and on postnatal treatment with testosterone. In addition to their state of permanent estrus, the androgenized rats present a picture similar to that of diestrus. Experimental data similar to those obtained in androgenized rats have been obtained with male animals. These alterations in the limbic system may be due to hormonal disequilibration consequent on androgenization, or possibly may be a direct effect of the postnatal treatment.

Resumen. Se ha estudiado el metabolismo oxidativo de amígdala e hipocampo en ratas en fase de estro, diestro y androgenizadas postnatalmente con testosterona. En estos animales desaparecen las variaciones observadas en animales cíclicos, presentando unos valores semejantes a los de diestro.

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Multiple Lactate Dehydrogenase Alleles in the Lizard *Agama stellio*

Agama stellio is probably the most conspicuous reptile of Israel. The species is widely distributed from the eastern Mediterranean region to Iraq[1]. Standard techniques of horizontal starch-gel electrophoresis were utilized to examine the mobilities of the lactate dehydrogenase (LDH) isozymes from 347 specimens of this species. We used buffer systems of pH 6.0[2], and 8.6[3]; and the histochemical staining procedure of Fine and Costello[4]. As among many vertebrates the LDH's of many lizards, including *Agama stellio*, are the products of two different genetic loci which may be termed H (heart) and M (muscle)[5,6]. The active enzyme is a tetramer and the products of the H and M loci can form hybrid tetramers. If the organism was homozygous at both loci, 5 isoenzymes could be seen on gel electrophoresis. An organism heterozygous at one of the two loci could express 15 isoenzymes[7].

We utilized red blood cells lysed with distilled water; and skeletal muscle and heart tissue from which the LDH was extracted not by the conventional method of tissue grinding but by soaking 1 volume of tissue in 4 volumes of 2% 2-phenoxyethanol in a 0.25 *M* sucrose solution[8].

Virtually all of the specimens were collected from areas administered by Israel (Table). The great majority had 5 equally spaced LDH bands in the red cell extracts. The anodally fasted migrating band predominated in heart tissue, the slowest band in skeletal muscle. At pH 8.6 all bands migrate toward the anode, at pH 6.0 the slow bands exhibit cathodal migration (Figure 1). No variation in electrophoretic mobility was seen among the 5-isoenzyme homozygous lizards.

There were 21 specimens that differed from the typical pattern, exhibiting, in all, 3 different electrophoretic phenotypes. All were 15 banded heterozygotes, with the heterozygosity involving the *H* locus[9]. The alleles were arbitrarily called LDH-H^n ('normal' or commonest), LDH-H^s ('slowest'), LDH-H^f ('fast'), and LDH-H^v ('very fast'). At pH 8.6 distinct differences could be seen between the mobilities of the heterozygotes *Hnf* and *Hnv*. At pH 6.0 the mobility appears tobe the same (Figure 1). The lower pH provides superior resolution of the expected 15 isoenzymes.

All the heterozygotes were concentrated in a relatively limited geographic area (Figure 2, Table). The allele H^v was found at all major collecting sites within the area encompassed by the presence of heterozygotes, but nowhere was it common (Table). Thus it is not surprising that no *Hvv* homozygotes were found. The other alleles were even rarer, and again homogzyotes would not have been expected. The alleles H^f and H^s were limited in distribution, the former to a single area in the Golan and the latter at 2 sites separated by a linear distance of about 50 km (Figure 2).

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Localities sampled, heart LDH genotypes and gene frequencies of *Agama stellio*

Map No.	Locality	No. of Specimens	Genotypes: *Hnn*	*Hnv*	*Hnf*	*Hns*	Gene frequencies (%) *Hn*	*Hv*	*Hf*	*Hs*
1	Sede Boqer region[a]	14	14				100	0	0	0
2	Arad	5	5				100	0	0	0
3	Be'er Sheva	19	19				100	0	0	0
4	Lahav	2	2				100	0	0	0
5	Jerusalem region[b]	35	35				100	0	0	0
6	Tel Aviv	44	44				100	0	0	0
7	Hadera	27	27				100	0	0	0
8	Haifa	23	23				100	0	0	0
9	Bet Alfa	73	66	2	0	5	95.2	1.4	0	3.4
10	Kare Deshe region[c]	43	35	7	0	1	90.7	8.1	0	1.2
11	Bar'am	6	4	2	0	0	83.3	16.7	0	0
12	Baniass region[d]	18	15	1	2	0	91.7	2.8	5.6	0
13	Nahariyya region[e]	34	33	1	0	0	98.5	1.5	0	0
Not shown on map										
	Sinai Peninsula (no precise locality)	3	3	0	0	0	100	0	0	0
	Southern Jordan	1	1	0	0	0	100	0	0	0
	Total	347	326	13	2	6				
Sex of heterozygotes[f]				4♂, 2♀	2♀	1♂				

[a] Includes 'En Avedat (2 spec.). [b] Includes Zova (16 spec.). Qiryat Anavim (18 spec.). [c] Includes Ammi'ad (8 spec.); Capernaum (1 spec.); Golani Road junction. (2 spec.) and 2 km N of Tiberias (2 spec.). [d] Includes Dan (3 spec.). [e] Includes Sa'ar (23 spec.) and Kabri (6 spec.). [f] Sex was determined by external characters. Sex of juveniles is unknown.

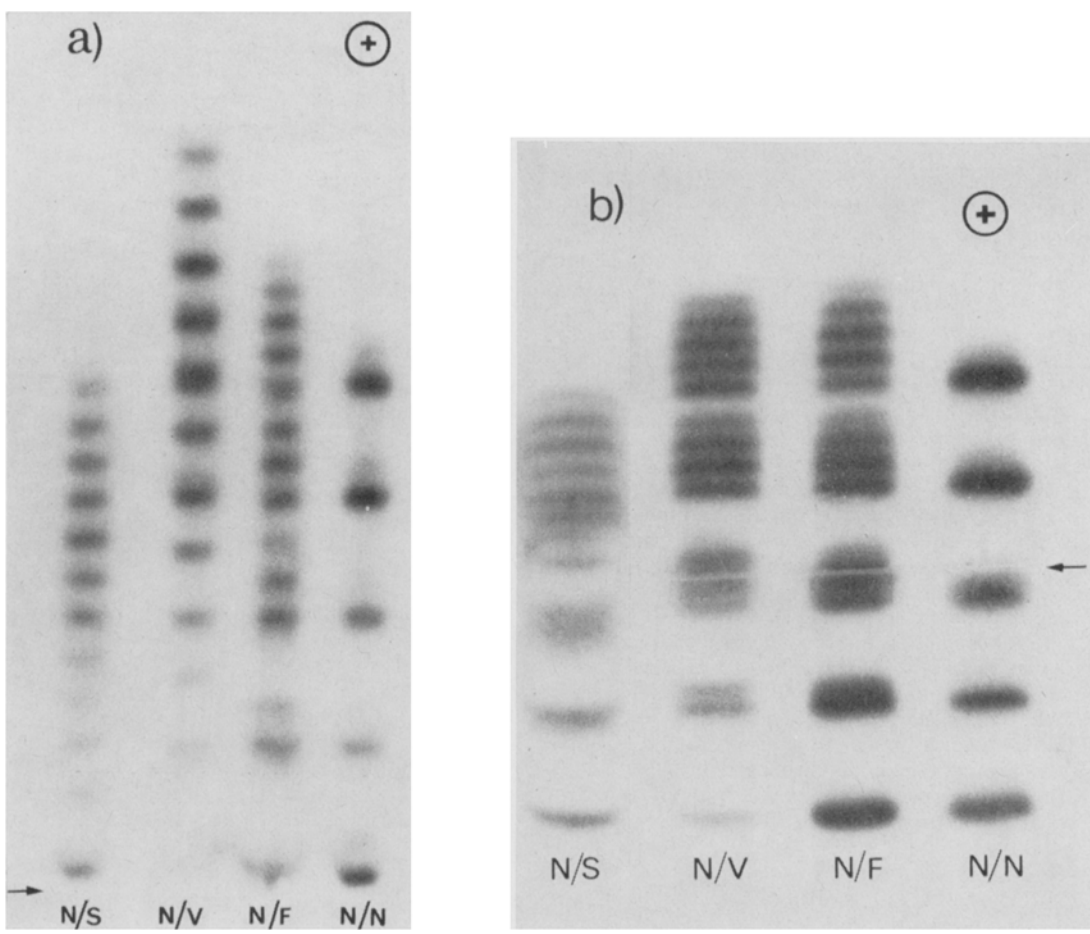

Fig. 1. Electrophoresis of hemolysed red blood cells stained for LDH. Arrow indicates origin; +, the anode. The 4 observed phenotype are shown, right to left *Hnn* homozygote (N/N); *Hnf* heterozygote (N/F); *Hnv* heterozygote (N/V); and *Hns* heterozygote (N/S). a) pH 8.(gel, clearly showing the 4 observed phenotypes. b) pH 6.0 gel utilizing same specimens as in a). Note absence of electrophoretic differenc between *Hnv* (N/V) and *Hnf* (N/F) but increased resolution of all the isozymes.

The common allele H^n and the variants were not equally expressed. It was clear on all gels that contained samples with *Hnf* or *Hnv* phenotypes that the n_4 tetramer was expressed more strongly than the v_4 or f_4 (Figure 1). This difference of expression was temperature independent, for gels incubated with the staining mixture at 4°, 20°, and 37° all had the same ultimate pattern, although the rate of staining was, of course, slower at the lower temperatures.

We are not in a position to determine the genetic or ecological significance of the LDH variation in the northern and eastern portion of Israel. One could argue with equal gusto that natural selection is maintaining the polymorphism, or that selection is relaxed permitting variability.

We believe that the single most striking observation is that only where the relatively rare allele H^v is established do we find yet another rare allele. Interestingly, a somewhat parallel case of LDH variation has been described for man. GIBLETT[10] reviewed genetic markers in human blood and dismissed LDH in a few short paragraphs because variation is so rare in man. Yet KRAUSS and NEELY[11] found 3 different variants of the *M* LDH locus in a relatively small sample from a restricted area. OHNO et al.[12] suggested that polymorphism might generate additional polymorphism, the new alleles being the resultants of intracistronic crossing over in a heterozygous parent. This might account for our unusual finding because once a second allele was established, other alleles could arise by cross-over.

To enter the realm of speculation, it seems probable that the gene products of alleles H^v and H^f differ by a single amino acid; that even in the complete absence of sequence data we can make an educated guess as to the nature of this difference; and that this difference could have arisen through intracistronic cross-over.

At pH 6.0 histidine, arginine, and lysine are all positively charged, whereas at pH 8.6 histidine is neutral. Thus substitution of an arginine or lysine codon in H^f from a histidine codon in H^v would account for the observation on gel-electrophoresis of identical mobility at pH 6.0, different mobility at pH 8.6.

Two of the codons for histidine (CAU and CAC) differ by only a single base pair from 2 of the codons for arginine (CGU and CGC). The codons for lysine are not so closely related to arginine or histidine. Whether by point mutation or intra-cistronic cross-over, by inference it appears that the products of alleles H^v and H^f differ in the substitution of arginine in H^f from histidine in H^v[13].

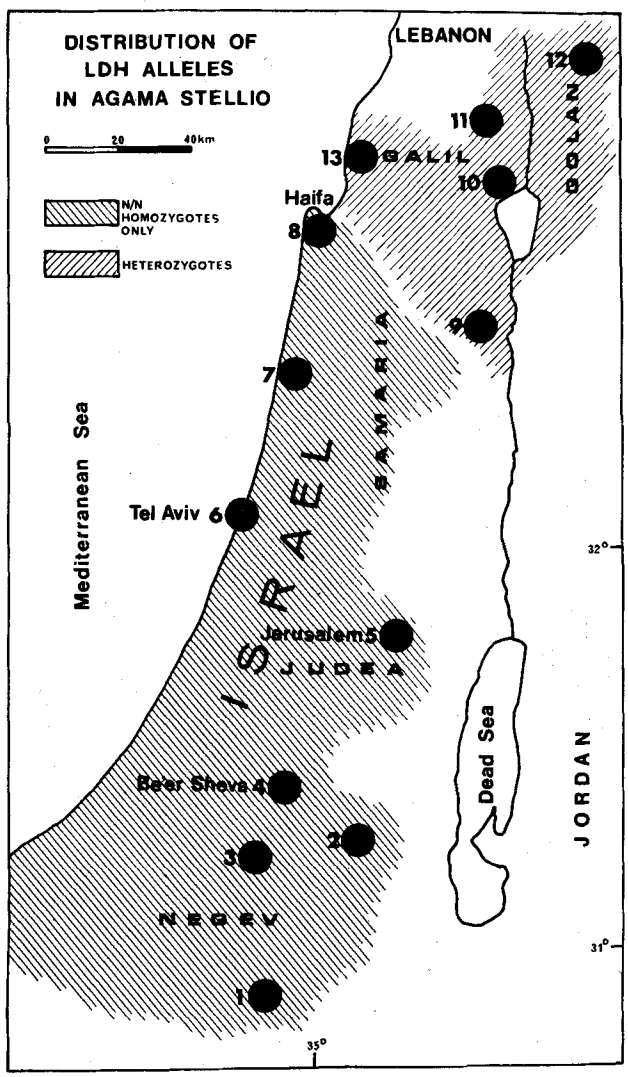

Fig. 2. Collecting localities of *Agama stellio*. Black circles are major collecting sites. Numbers correspond to localities listed in the Table. Hatching shows areas of H LDH homozygosity and heterozygosity. Localities 1–8 included only *Hnn* (N/N) homozygotes (169 specimens). Localities 9–13 included all the heterozygotes that were found. Allele LDH-H^v was found at sites 9–13; LDH-H^s at 9 and 10; LDH-H^f at 12. Areas in white imply lack of specimens, not absence of the species.

Zusammenfassung. Es wurde das gehäufte, geographisch begrenzte Auftreten multipler Allele für LDH-H, das bei den bisher untersuchten Organismen nur selten Varianten zeigt, nachgewiesen. Ebenso gelang die Identifizierung von elektrophoretischen Varianten in Abhängigkeit vom pH.

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