methods. It seems likely that the trypsin method is useful for karyotype analysis in other amphibia. Recently, sex chromosomes were discovered in 2 anuran species by using other banding techniques[12,13]. Investigation is under way in our laboratory on sexual differences in the bandings.

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Clinal variation at the peptidase-1 (*Pept*-1) locus in natural populations of *Drosophila subobscura*[1]

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Summary. The allele frequencies for the peptidase-1 (*Pept*-1) gene in 8 natural populations of *Drosophila subobscura* situated along a north-south transect through the distribution range of the species were determined. The occurrence of a cline along the transect studied is discussed in relation to the mechanisms of maintenance of the *Pept*-1 genetic polymorphism.

The adaptive significance of protein polymorphisms in natural populations of diploid organisms is still a much-debated point in evolutionary genetics[3,4]. It is not clear whether such genetic variability is maintained by some kind of balancing selection or is completely neutral and only subject to random genetic drift. Moreover, there are now numerous data available showing that simple electrophoretic techniques reveal much less of the genetic variability at the protein level than actually exists[5].

The present paper deals with the allele frequency variation of the peptidase-1 (*Pept*-1) locus in 8 natural populations of *Drosophila subobscura*. The gene coding for *Pept*-1 was found to be on chromosome 0 of *D. subobscura*[6]. The different collection sites are located approximately along a north-south line across the distribution range of the species (see table). The present investigation is part of a more extensive project for the study of the genetic composition of natural populations of *D. subobscura*[7,8].

The 14% starch horizontal gel electrophoresis conditions used were as follows. Electrode buffer: 0.18 M Tris, 0.1 M boric acid, 0.004 M EDTA 2 Na; Gel buffer: 1 in 4 dilution of bridge buffer. The final pH was 8.6. The run was at 250 V/70 mA/5 h. The staining mixture, modified by Harris and Hopkinson[9], utilized for 1 gel contained 0.1 M Na_2HPO_4 adjusted to pH 7.5 with 5 ml 0.1 M KH_2PO_4; 10 mg O-dianisidine; 10 mg Snake venom L-aminoacid oxidase; 10 mg peroxidase grade II (100 U/mg); 20 mg L-phenylalanyl-L-leucine; 10 mg $MnCl_2$; 20 ml agar (approximately 0.8% in 0.1 M phosphate buffer). The gels were stained at 37 °C. Enzyme activity was indicated by dark yellow-brown staining bands. The allozyme pattern of the *Pept*-1 locus found in the present study can easily be explained by the presence of 4 alleles (0.20, 0.40, 1.00, and 1.60). Homozygotes exhibit only single bands, heterozygotes 3 bands, as previously demonstrated for the dipeptidase-A locus in *D. melanogaster*[10].

The alleles found, their frequencies and the degrees of heterozygosity in the population samples studied are given in the table. Three common (0.40, 1.00, and 1.60) and 1 rare (0.20) active *Pept*-1 alleles were observed. No inactive

Allele frequencies (%) at the *Pept*-1 locus in 8 populations of *Drosophila subobscura*

Population	Location[a] Lat. (N)	Long.	Sample size	Allele 0.20	0.40	1.00	1.60	Degree of heterozygosity
1. Sunne (Sweden)[b]	59.50	13.09 E	9	0	33.3±16	66.7±16	0	44.4
2. F. Augustus (Scotland)[c]	57.10	4.41 W	191	0.6±1	48.3± 4	47.2± 4	3.9±2	53.9
3. Tübingen (Germany)[d]	48.31	9.02 E	215	0.4±1	43.1± 3	51.6± 2	4.9±1	54.6
4. Zernez (Switzerland)[b]	46.43	10.05 E	12	0	66.7±14	25.0± 3	8.3±8	48.6
5. Formia (Italy)[c]	41.15	13.37 E	157	0	70.8± 4	27.1± 4	2.1±1	42.5
6. Ponza (Italy, Island)[b]	40.54	12.58 E	19	0	78.9± 9	21.1± 9	0	33.3
7. Cinisi (Italy, Sicily)[b]	38.08	13.18 E	14	0	64.3±13	35.7±13	0	45.9
8. Bizerte (Tunesia)[b]	37.17	9.52 E	11	0	81.8±12	18.2±12	0	29.8
							Average:	44.6

[a]For further details see Pinsker and Sperlich[7] and Sperlich et al.[8]. Seasonal and altitudinal differences can be neglected since they are only weakly expressed in *D. subobscura;* [b]data from balanced lethal stocks; [c]data from balanced lethal stocks and isofemale lines; [d]data from balanced lethal stocks (14), isofemale lines (82), and a natural population (119).

alleles have been recognized so far. All populations proved polymorphic for at least 2 *Pept*-1 alleles. The average degree of heterozygosity of the *Pept*-1 gene (=44.6) is clearly above the average for all loci calculated for this species by Pinsker and Sperlich[7] (13.7; 17 loci in 7 populations) and Marinkovic et al.[11] (11.4; 28 loci in 1 population). Hence, the *Pept*-1 gene must be considered one of the most polymorphic in *D. subobscura* (like *Aph*-3, *Hk*-1, and *Lap*-4[7]).

From the table it is further seen that a general and quite strong cline exists for the frequencies of the *Pept*-1 alleles 0.40 and 1.00 moving from north to south. A frequency difference of about 50% is visible if the 2 most peripheral populations, Sunne and Bizerte, are compared. The correlation between the frequencies of the allele 0.40 and the geographic latitudes is statistically highly significant ($r = -0.874$ with 6 d.f., $p < 0.01$).

The cline in *Pept*-1 alleles of *D. subobscura* which we observed in the present survey appears to be of special interest since such clinal changes of allozyme allele frequencies are not common in natural populations of other *Drosophila* species[12]; yet some cases have been reported[13]. Our finding can hardly be explained by genetic drift between geographically separated populations alone. It might rather be connected with the strong inversion polymorphism present in this species. Since gene arrangements are not thought to be selectively neutral and since the frequencies of a number of inversions on chromosome 0 of *D. subobscura* follow geographically a quite similar pattern to that observed for the alleles of the *Pept*-1 locus, coselection due to tight linkage seems a plausible explanation. This assumption is sustained directly by our data and the inversion frequency data if the results from neighbouring 'continental' populations and 'island' populations (populations 1 vs 2, 5 vs 6, and 8 vs 7) are compared. The frequencies of the *Pept*-1 alleles in the 'island' populations always differ somewhat from the 'continental' populations. Such a differentiation is also found for the frequencies of the various chromosomal arrangements of chromosome 0 of *D. subobscura*[14].

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Effects of prednisolone and butyrate on agglutinability of HeLa cells by concanavalin A[1]

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Summary. Agglutinability by concanavalin A was measured with $HeLa_{65}$ cells grown with prednisolone or sodium butyrate, 2 compounds that increase the activity of the carcinoplacental form of alkaline phosphatase, an enzyme localized in membranes. Prednisolone enhanced concanavalin A agglutination approximately 3-fold while sodium butyrate had no effect.

HeLa, a cervical carcinoma cell line, has been extensively characterized and provides a good model for studies of gene expression. Both prednisolone (Δ^1-hydrocortisone), a steroid hormone with potent glucocorticoid activity, and sodium butyrate, a 4-carbon aliphatic monocarboxylate, alter gene expression in $HeLa_{65}$ cells. Both increase the activity of the carcinoplacental form of alkaline phosphatase[2-6], an enzyme localized in membranes. Butyrate induces synthesis of human chorionic gonadotropin[7-10] whereas prednisolone does not[10]. Prednisolone also causes a morphological alteration with flattening of $HeLa_{65}$ cells and an increase in the amount of cytoplasm[2,5] whereas butyrate causes $HeLa_{65}$ cells to become more spindle shaped and fibroblastic[5].

In the present study, $HeLa_{65}$ cells were grown with either prednisolone or butyrate and the effect on agglutinability