A SERUM AMYLASE POLYMORPHISM IN POPULATIONS OF THE BRUSH-TAILED POSSUM *TRICHOSURUS VULPECULA**

By R. M. HOPE† and D. J. FINNEGAN†‡

During the last ten years, the techniques of gel electrophoresis have revealed a large amount of heritable biochemical variation in populations of many organisms. Lewontin and Hubby (1966) have estimated that on the average 30% of all loci are heterozygous in natural populations of *Drosophila pseudoobscura*, and similar estimates have been obtained for other species of *Drosophila* (O'Brien and MacIntyre 1969) and for man (Harris 1966). The ecological and evolutionary significance of this variation is as yet almost completely unknown. An experimental approach to this problem is to study the nature and extent of variation within and between samples from subpopulations of a single species, and to relate this variation to environmental factors and breeding structure. This approach has indicated the nature of the selection on several morphological polymorphisms (Sheppard 1959; Kettlewell 1965) but with the exception of the sickle cell haemoglobin polymorphism in man (Allison 1954) it has provided little evidence concerning the factors affecting biochemical polymorphisms.

The results reported below are part of an extensive study of several biochemical polymorphisms in the Australian marsupial *Trichosurus vulpecula* (Kerr), the brush-tailed possum.

We have found two electrophoretic variants of a serum amylaseisoenzy me. The two variants have been found segregating in six of the populations studied. We also present evidence to support the conclusion that the variable serum amylase band is due to enzyme produced by the pancreas.

Experimental

The samples were collected by cardiac puncture from anaesthetized animals. Electrophoresis was carried out in vertical water-cooled gel trays (30 cm by 15 cm by 5 mm) with sample slots 7·5 cm from the cathode end. The gels were 5% acrylamide [1·5 g *N,N'*-methylenebisacrylamide, 25 g acrylamide, 500 ml buffer, 0·5 ml *N,N,N',N'*-tetramethylethylenediamine, 8 ml 5% $(NH_4)_2S_2O_8$ solution], and the discontinuous Tris–citrate buffer system of pH 8·6 described by Ashton and Braden (1961) was used. The gels were stained by the method described by Boettcher and de la Lande (1969*a*, 1969*b*) for human salivary amylase modified in that the starch solution was buffered to pH 6·0 with 0·1M phosphate buffer.

Results

The three serum amylase patterns observed are illustrated in Figure 1. The most common pattern consisted of two zones of amylase activity. The more anodal was a single strong band. The second region nearer the origin was not present in all samples. When it was present it could often, but not always, be resolved into two, three, or four bands of activity. Two other phenotypes with respect to the most

* Manuscript received August 25, 1969.

† Department of Genetics, University of Adelaide, G.P.O. Box 498D, Adelaide, S.A. 5001.

‡ Department of Molecular Biology, Kings Building, West Mains Road, Edinburgh 9, Scotland.

anodic band were found, one with a single band with slightly faster mobility towards the anode, and the other with both the fast and slow bands together. We have called the faster form type A, the slower form type B, and the double-banded phenotype type AB (Fig. 1).

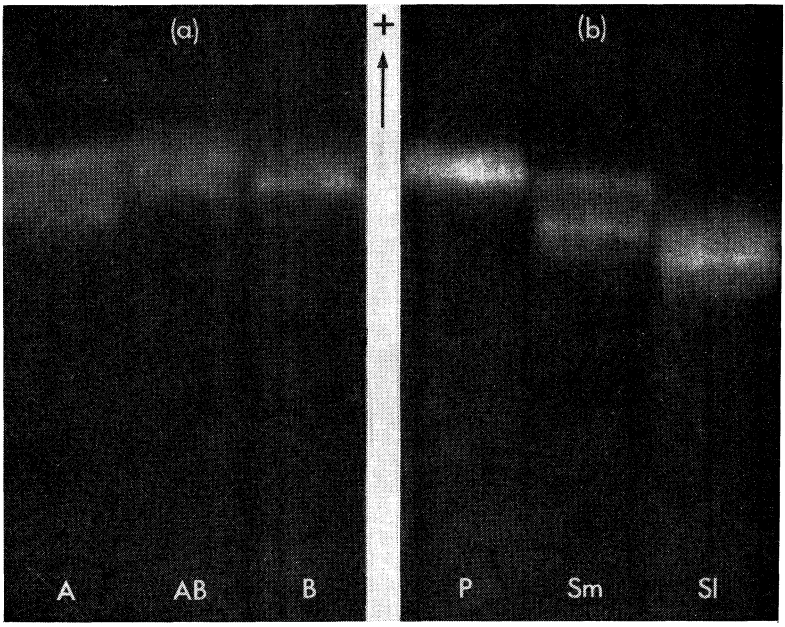

Fig. 1.—(*a*) The three serum amylase phenotypes of *T. vulpecula* A, AB, and B. Notice that the slower zone is absent from the AB and B samples. (*b*) Amylase bands of pancreatic extract (P), serum (Sm), and saliva (Sl) from an animal of type B.

Kamarýt and Laxovà (1965) have shown that human serum amylase consists of isoenzymes identical to those found in saliva and pancreatic extracts. Nielsen (1969) has found that the serum of the bank vole *Clethrionomys glareola* has amylase isoenzymes from these sources as well as other bands whose origins are unknown and we have unpublished evidence that the serum of the marsupial mouse *Sminthopsis crassicaudata* contains modified pancreatic amylase.

Samples of saliva and pancreatic extracts were obtained as described by Sick and Nielsen (1964) and these were analysed on gels. The saliva samples yielded a series of three to four bands which were best resolved with the discontinuous Tris–citrate buffer system of pH 7·7 described by Ferguson and Wallace (1961). The pancreatic extract had one strong amylase band. Comparison of the serum, saliva, and pancreatic extract of one animal (Fig. 1) showed that the serum amylase isoenzymes could be derived from a combination of the salivary and pancreatic enzyme, with the variable serum band corresponding to that from the pancreas. The initial pancreatic extracts were made from an animal with type B serum amylase. To test the hypothesis that the variable isoenzyme is produced by the pancreas we analysed extracts from individuals with type A and AB sera. The former had a single fast band and the

latter two bands, and again they were of the same mobility as the corresponding serum bands. Thus we have concluded that the serum variants reflect variations of the enzyme produced by the pancreas.

The sera of animals captured in localities throughout Australia have been analysed and the results are given in Table 1.

TABLE 1

FREQUENCIES OF THE THREE AMYLASE PHENOTYPES FOUND IN EACH POPULATION, TOGETHER WITH THE FREQUENCY OF THE A GENE

The number beside each population refers to the number of the population in Figure 2

Population	No. of Animals	Phenotypic Frequencies			Frequency of A Gene	S.E.
		A	AB	B		
1. Perth	32	17	11	4	0·70	0·018
2. Louth Bay	25	0	0	25	—	—
3. Flinders Chase	44	0	0	44	—	—
4. Penneshaw	33	0	0	33	—	—
5. Adelaide	117	1	31	85	0·22*	0·011
6. Nildottie	42	1	13	28	0·18	0·042
7. Coleraine	18	1	9	8	0·31	0·025
8. Melbourne	24	0	0	24	—	—
9. Urana	49	0	7	42	0·07	0·026
10. Canberra	18	0	0	18	—	—
11. Sydney	60	1	7	52	0·075	0·024
12. Darwin	5	0	0	5	—	—
13. Cairns	5	0	0	5	—	—

* Calculated from incomplete family data as described by Finney (1948).

TABLE 2

INCOMPLETE FAMILY DATA PERTAINING TO THE INHERITANCE OF THE SERUM AMYLASE VARIANTS

0, result expected but not observed; —, result not expected and not observed

Mother	Phenotypic Frequencies in Offspring			Totals
	A	AB	B	
A	0	1	—	1
AB	0	8	5	13
B	—	5	39	44
Totals	0	14	44	58

Discussion

The simplest way in which electrophoretic variants of a protein can be inherited is that they are controlled by allelic genes without dominance. Ashton (1965) has shown this to be the case for serum amylase variants in cattle and it would seem

reasonable to suggest that the same is true in *T. vulpecula*. Mother–offspring data for 116 individuals are shown in Table 2. These data do not contradict this hypothesis, but neither can they be said to confirm it. However, these results have been analysed on the basis of this mode of inheritance.

The samples from the six populations in which the A and B variants are segregating have been tested for agreement with random-mating expectations by means of 2×2 contingency tables. Exact probabilities were used when the smallest expected value was less than 5. The Adelaide data consist almost entirely of mother–offspring pairs and the analysis was performed on both the mother and offspring results separately. In no case was there a significant deviation from expectation and thus the populations may be regarded as mating at random with respect to serum amylase type.

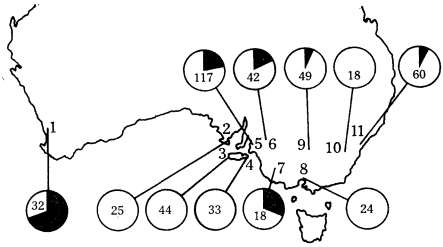

Fig. 2.—Map showing the frequencies of the A (black shading) and B (unshaded) genes in the various populations. The numbers within each circle are the numbers of animals sampled. The other numbers are the locations of populations as given in Table 1.

Gene frequencies have been calculated for these six populations, assuming a single locus model and they are shown in Table 1. The gene frequency from the Adelaide incomplete family data was estimated by the method of Finney (1948). Figure 2 shows the geographical location of each population together with its corresponding gene frequency.

Due to the very low gene frequency of the A gene in most populations, the gene frequencies could not be compared by approximation to the normal distribution. Instead, populations were compared pairwise using 2×2 contingency tables, to test for association of the number of A genes (2A+AB) and the number of B genes (2B+AB) with population. It was found that the populations form three groups: (1) Perth, (2) Adelaide, Nildottie, and Coleraine, and (3) Urana and Sydney, between which the gene frequency differs significantly at the 5% level of probability.

The use of Stevens' table (Table VIII, Fisher and Yates 1963) shows that we cannot, at the 5% level, exclude the possibility that the A gene occurs in the Melbourne and Canberra populations with the frequency with which it is found in Sydney and Urana.

In order to see whether or not selection, with respect to the amylase variants, was acting between conception and the age at which pouch young could first be bled, we have compared the gene frequencies of the Adelaide mothers with those of their offspring. There was no significant difference.

This study has revealed a serum amylase polymorphism in populations of *T. vulpecula* from several widely scattered regions of Australia. With the exception of Perth the A variant is the rarer form and its frequency varies considerably between populations. However, we have been unable to find within the population data any evidence to indicate what factors are maintaining the polymorphism. We suggest that such evidence might best be sought by an intensive ecological study of one of the polymorphic populations. The population data do help to identify the different populations of this species, and it will be of interest to compare these differences between populations with any differences which may be revealed by further study of other polymorphic systems.

Acknowledgments

We are indebted to Dr. M. J. Mayo and Dr. D. L. Hayman for their assistance, and we should like to acknowledge the following for their help in obtaining blood samples or for assisting in field collections: Mr. H. Butler, joint collector for the Western Australian Museum and the American Museum of Natural History, Professor G. B. Sharman, Professor E. C. Horn, Dr. J. Kirsch, Dr. J. A. Thomson, and Messrs J. Calaby, L. McLean, N. Morley, and R. Scollay.

References

Allison, A. C. (1954).—*Br. Med. J.* **1**, 290.
Ashton, G. C. (1965).—*Genetics, Princeton* **51**, 431.
Ashton, G. C., and Braden, A. W. H. (1961).—*Aust. J. biol. Sci.* **14**, 248.
Boettcher, B., and de la Lande, F. A. (1969*a*).—*Aust. J. exp. Biol. med. Sci.* **47**, 97.
Boettcher, B., and de la Lande, F. A. (1969*b*).—*Analyt. Biochem.* **28**, 510.
Ferguson, K. A., and Wallace, A. L. C. (1961).—*Nature, Lond.* **190**, 629.
Finney, D. J. (1948).—*Heredity, Lond.* **2**, 199.
Fisher, R. A., and Yates, F. (1953).—"Statistical Tables for Biological, Agricultural and Medical Research." 4th Ed. (Oliver and Boyd: Edinburgh.)
Harris, H. (1966).—*Proc. R. Soc. Lond.* B **164**, 298.
Kamarýt, J., and Laxovà, C. (1965).—*Humangenetik* **1**, 579.
Kettlewell, H. B. D. (1965).—*Science, N.Y.* **148**, 1290.
Lewontin, R. C., and Hubby, J. L. (1966).—*Genetics, Princeton* **54**, 595.
Nielsen, J. T. (1969).—*Hereditas* **61**, 400.
O'Brien, S. J., and MacIntyre, R. J. (1969).—*Am. Nat.* **103**, 97.
Sheppard, P. M. (1959).—*Cold Spring Harb. Symp. Quant. Biol.* **24**, 131.
Sick, M., and Nielsen, J. T. (1964).—*Hereditas* **51**, 291.