

A LATITUDINAL CLINE OF HAEMOGLOBINS IN A BIVALVE MOLLUSC

A. K. O'GOWER and PATRICIA I. NICOL

School of Biological Sciences, University of New South Wales

Received 28.xii.67

1. INTRODUCTION

THE selective advantages of morphological polymorphisms have been demonstrated for many animals (Cain and Sheppard, 1954; Dice, 1947; Ford, 1964) and, although biochemical polymorphisms have also been found in a wide range of animals (Allison, 1964; Cooper and Sharman, 1964; Crawford, 1966; Evans *et al.*, 1958; Manwell and Baker, 1963), the physiological advantages of fewer polymorphisms are known (Allison, 1964; Dawson and Evans, 1965; Manwell and Schlesinger, 1966; Woolf, 1954). Polymorphisms often form clines, which may either follow a gradient of environmental factors, abiotic (Kettlewell and Berry, 1961) or biotic (Brower and Brower, 1962), or the cline may form either a mosaic which is associated with discontinuous environmental factors (Cain and Sheppard, 1954) or a mosaic whose origin is historical (Mourant *et al.*, 1958).

The occurrence of a haemoglobin polymorphism in a population of *Anadara trapezia* (Deshayes) from Gunnamatta Bay, Sydney (Nicol and O'Gower, 1967), suggested studies might profitably be made on this polymorphism in other populations on the south-eastern coastline of Australia.

2. METHODS

Samples of *A. trapezia* were collected from seven populations along the south-eastern coast of Australia (fig. 1) and were held in aerated aquaria until they could be bled. Haemoglobin solutions were prepared and subjected to electrophoresis as described by Nicol and O'Gower (1967). The resultant patterns included two or three major bands, plus "trace" bands. The distribution of the major bands throughout the sample suggested a single, allelic, genetic mechanism. This hypothesis was tested by a chi-square test for "goodness-of-fit" of the gene frequencies of each of the seven samples of *A. trapezia* to the expected Hardy-Weinberg frequencies. The regression of gene frequencies for the five populations from Lake Burrill to Southport against latitudinal distance was calculated. The 95 per cent. confidence interval for this regression included the gene frequencies of the populations at Mallacoota Inlet and Point Vernon. Therefore, the regression of the gene frequencies of all seven populations against the latitudinal distance was calculated.

3. RESULTS

The major electrophoretic haemoglobin bands labelled Hb¹, Hb^{2a} and Hb^{2b} occurred in three patterns (patterns 1, 2 and 3; see fig. 2).

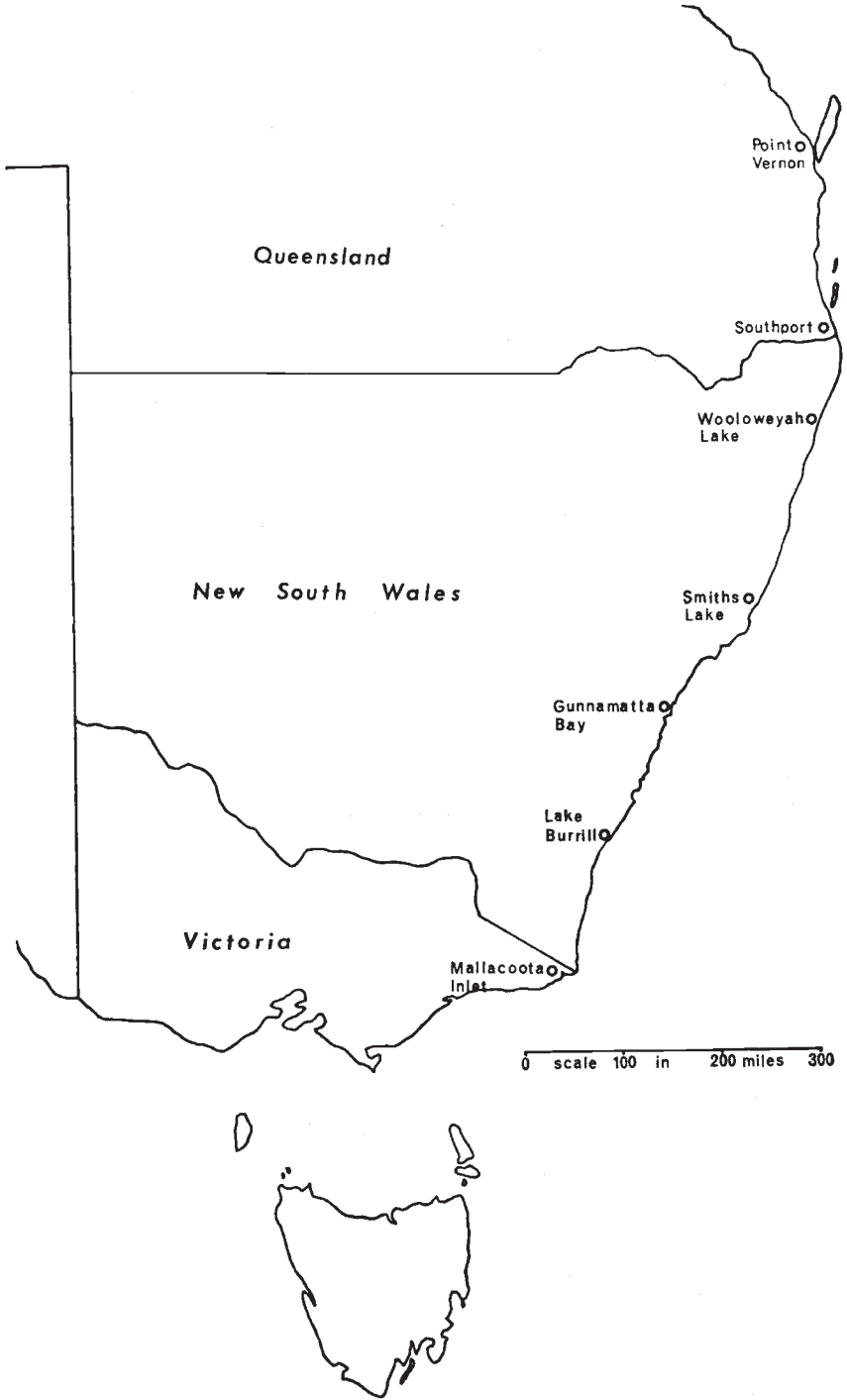


FIG. 1.—Sampling sites for populations of *A. trapezia* on the south-eastern coastline of Australia.

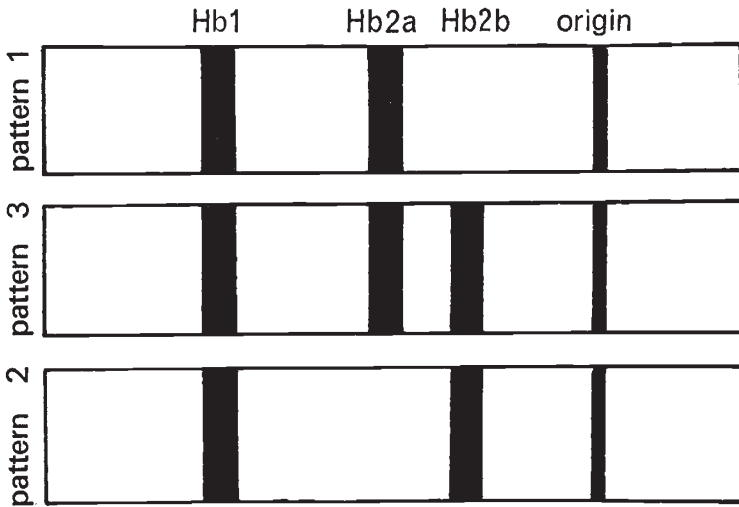


FIG. 2.—The three patterns of the major electrophoretic haemoglobin bands of *A. trapezia*.

The frequency distribution of the patterns and the corresponding gene frequencies of the seven different samples of *A. trapezia* are shown in table 1

TABLE 1

Frequency distribution and gene frequencies of haemoglobin electrophoresis patterns of populations of A. trapezia, chi-square values and probabilities for "goodness-of-fit" to the Hardy Weinberg Law

Locality	Pattern			χ^2	P	Gene frequency
	1	2	3			
Mallacoota Inlet	4	155	41	0.433	0.50	0.1225
Lake Burrill	0	162	30	1.385	0.75	0.078
Gunnamatta Bay	8	113	66	0.179	0.25	0.219
Smiths Lake	21	156	129	0.675	0.60	0.279
Woolweyah Lake	20	44	51	0.608	0.60	0.396
Southport	20	25	56	1.261	0.75	0.475
Port Vernon	19	40	59	0.126	0.25	0.411

with the corresponding chi-square values and probabilities for their "goodness-of-fit" to the Hardy-Weinberg Law. The regression of the gene frequencies for the seven sampled populations against latitudinal distance is $\bar{Y} = 0.091407 + 0.000418X$ (see fig. 3). Note that the scale on the X axis is roughly 10^3 times the scale on the Y axis. Hence the slope of the regression is at a definite angle to the X axis.

4. DISCUSSION

The "goodness-of-fit" between the observed frequency distribution of haemoglobin electrophoresis patterns of seven populations of *A. trapezia* and the expected Hardy-Weinberg frequencies (table 1), strongly supports the hypothesis that a single allele controls the haemoglobin polymorphism (band 2 of fig. 2) (Nicol and O'Gower, 1967). On this hypothesis the relationship between gene frequency for haemoglobin morph (table 1) and latitudinal

distance (fig. 1) is given by the regression $Y = 0.091407 + 0.000418X$ (fig. 3); that is, there is a latitudinal cline of haemoglobin polymorphism in *A. trapezia*.

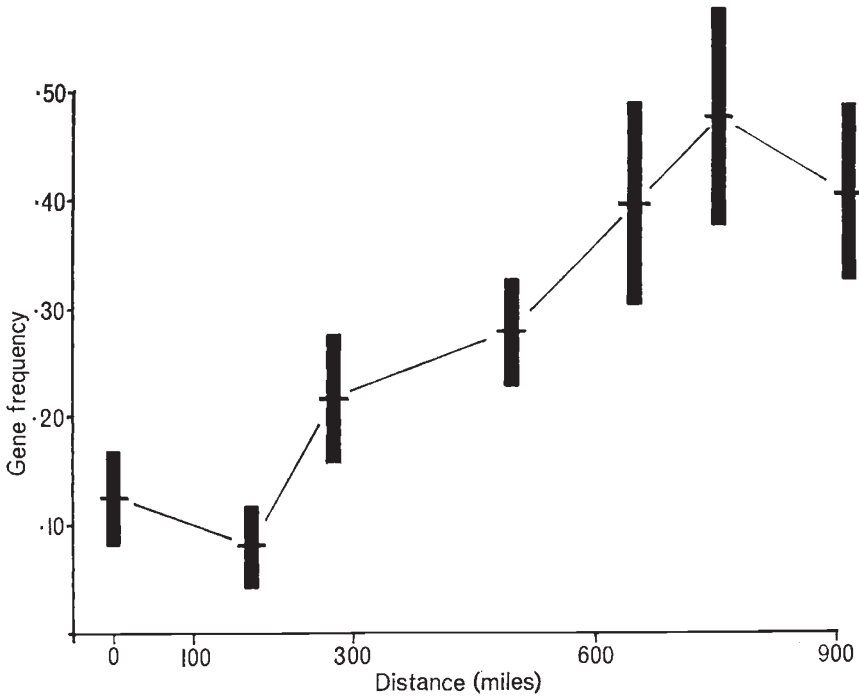


FIG. 3.—Regression of gene frequency on latitudinal distance for seven populations of *A. trapezia* on the south-eastern coastline of Australia (mean and 95 per cent C.I.).

The reversals in gene frequencies at each extreme of the cline (fig. 3), although not significant ($0.10 > P > 0.05$) are probably ecologically significant, for the straight part of the regression in fig. 3, between Southport and Lake Burrill (fig. 1) is correlated with the East Australian Current, which follows the coastline from Brisbane, between Point Vernon and Southport, to Eden, between Lake Burrill and Mallacoota Inlet (Hamon, 1961). The northern extreme of the cline, Point Vernon, lies in the complex tidal system of the Great Barrier Reef (Maxwell, 1968) which is characterised by current oscillations and vortices (Woodhead, personal communication). The southern extreme of the cline, Mallacoota Inlet, is marginally associated with the oscillating Bass Strait Current (Rochford, 1960). That these three current systems, the Great Barrier Reef tidal current, the East Australian Current and the Bass Strait Current differ ecologically is reflected in the littoral faunas of these three regions (Bennett and Pope, 1953, 1960; Endean *et al.*, 1956).

Where the cline in gene frequencies is linear there is an obvious correlation between linearity and the East Australian Current. As the East Australian Current originates in the warm, tropical waters of the Coral Sea, passes southwards from near Southport to beyond Lake Burrill (fig. 1) and

then moves eastwards towards New Zealand (Hamon, 1961), it is apparent that the East Australian Current will form a temperature gradient along the east Australian coastline. Hence, the gradient in the gene frequencies of the electrophoretic haemoglobin patterns is also correlated with a water temperature gradient. Such a cline could be correlated with the physiological advantages of the haemoglobin morph relative to temperature, or the "haemoglobin genes" could be linked to other genes conferring other selective advantages on the animals. Consequently, it might be expected that the cline will reflect a differential either in the survival of the planktonic larval stages or the benthic adults to temperature (Kinne, 1964; Lewis, 1964; Moore, 1958), or in the physiological advantages of the genotypes to temperature. These physiological advantages may either directly influence the population, *e.g.* gonad maturation (Lewis, 1964), or such advantages may themselves enhance the abilities of the genotypes to survive other adverse environmental parameters, *e.g.* the effect of temperature on metabolic activity under osmotic stress (Kinne, 1964; Moore, 1958).

As haemoglobin is a respiratory pigment, the latitudinal gradient in the haemoglobin polymorphisms of *A. trapezia* could be correlated with the oxygen-binding properties of the haemoglobin morphs in relation to temperature (Manwell and Schlesinger, 1966; Naughton *et al.*, 1963). However, as *A. trapezia* is restricted to the shallow, quieter waters of bays, inlets and estuaries, where insolation could be great and where limited periods of osmotic stress could be encountered, and as the oxygen-carrying capacity of water varies with both temperature and salinity (Kinne, 1964; Richards, 1957), the relationship between the environment and the oxygen-binding capacities of the three haemoglobin morphs could be quite complex. Studies on the effects of temperature, carbon dioxide, pH and ionic environment on the oxygen dissociation curves of the three haemoglobin types are at present being made to determine whether the latitudinal cline is correlated with the oxygen-binding properties of the three haemoglobin morphs of *A. trapezia*.

As the East Australian Current flows southwards, any gene flow between populations of *A. trapezia* must also be in a southwards direction, but, as each population is restricted to tidal estuarine bays, some inbreeding must occur. Hence the gene frequency of each population is the end-result of genotype advantage to some measure of water temperature and limited southwards gene flow between populations.

It can therefore be postulated that populations in regions of oscillating currents should have similar gene frequencies due to omnidirectional gene flow. Populations in the Great Barrier Reef tidal system and in the Bass Strait Current system are at present being sampled for determination of their gene frequencies.

That the gene frequencies of populations can vary with time over succeeding generations is apparent (Petras, 1967), and as the gene frequencies of the populations in the cline of *A. trapezia* were determined from individuals of varying ages, this effect must also be studied. As it is apparent that sample size influences the variance of any gene frequency determination, the optimum sample size should also be determined. The influence of these two variables on gene frequency are at present being studied. However, in this investigation the populations of *A. trapezia* were so far apart that sampling variability could not greatly affect the cline in gene frequencies, but it is apparent that the 95 per cent. confidence intervals do overlap for some adjacent popula-

tions. Also in progress are studies on the gene frequencies of populations of *A. trapezia* from localities between those selected in the present study (fig. 1) to determine the effect of distance on gene frequency.

5. SUMMARY

1. The "goodness-of-fit" of haemoglobin polymorphisms of seven populations of *A. trapezia* to the Hardy-Weinberg Law indicates that this polymorphism is very probably controlled by a single allele.

2. The regression of gene frequencies for the haemoglobins on latitudinal distance follows a latitudinal cline which is mainly correlated with the East Australian Current.

3. The reversals in gene frequencies at the northern and southern extremes of the cline are probably correlated with the Great Barrier Reef tidal current system and the Bass Strait Current system respectively.

Acknowledgements.—Mrs McKeon is thanked for kindly collecting the population of *A. trapezia* from Point Vernon, Queensland.

6. REFERENCES

- ALLISON, A. C. 1964. Polymorphism and natural selection in human populations. *Cold Spring Harb. Symp. quant. Biol.*, 24, 137-149.
- BENNETT, ISOBEL, AND POPE, ELIZABETH C. 1953. Intertidal zonation of the exposed rocky shores of Victoria together with a rearrangement of the biogeographical provinces of temperate Australian waters. *Aust. J. Mar. Freshw. Res.*, 4, 105-159.
- BENNETT, ISOBEL, AND POPE, ELIZABETH C. 1960. Intertidal zonation of the exposed rocky shores of Tasmania and its relationship with the rest of Australia. *Aust. J. Mar. Freshw. Res.*, 11, 182-221.
- BROWER, L. P., AND BROWER, J. VAN Z. 1962. The relative abundance of model and mimic butterflies in natural populations of the *Battus philenor* mimicry complex. *Ecology*, 43, 154-158.
- CAIN, A. J., AND SHEPPARD, P. M. 1954. Natural selection in *Cepaea*. *Genetics*, 39, 89-116.
- COOPER, D., AND SHARMAN, G. B. 1964. Transferrin variation in kangaroos. *Nature*, 203, (4949), 1094.
- CRAWFORD, M. H. 1966. Hemoglobin polymorphism in *Macaca nemestrina*. *Science*, 154, 398-399.
- DICE, L. R. 1947. Effectiveness of selection by owls of deer mice (*Peromyscus maniculatus*) which contrast in colour with their background. *Contrib. Lab. Vertebr. Biol. Univ. Mich.*, 34, 1-20.
- DAWSON, T. J., AND EVANS, J. V. 1965. Effect of hemoglobin type on the cardiovascular system of sheep. *Am. J. Physiol.*, 209, 593-598.
- ENDEAN, R., KENNY, R., AND STEPHENSON, W. 1956. The ecology and distribution of intertidal organisms on the rocky shores of the Queensland mainland. *Aust. J. Mar. Freshw. Res.*, 7, 193-253.
- EVANS, J. V., HARRIS, H., AND WARREN, F. L. 1958. The distribution of haemoglobin and blood potassium types in British breeds of sheep. *Proc. R. Soc. B*, 149, 249-262.
- FORD, E. B. 1964. *Ecological Genetics*. Methuen, London.
- HAMON, B. V. 1961. The structure of the East Australian Current. *Aust. C.S.I.R.O. Div. Fish. Oceanogr. Tech. Paper 11*. 11 pp., 3 figs., 1 table.
- KETTLEWELL, H. B. D., AND BERRY, R. J. 1961. The study of a cline. *Heredity*, 16, 403-414.
- KINNE, O. 1964. The effects of temperature and salinity on marine and brackish water animals. II. Salinity and temperature-salinity relations. *Ann. Rev. Oceanogr. mar. Biol.*, 2, 281-339.
- LEWIS, J. R. 1964. *The Ecology of Rocky Shores*. English Universities Press, London.
- MANWELL, C., AND BAKER, C. M. A. 1963. A sibling species of sea cucumber discovered by starch-gel electrophoresis. *Comp. Biochem. Physiol.*, 10, 39-53.
- MANWELL, C., AND SCHLESINGER, C. V. 1966. Polymorphism of turtle haemoglobin and geographical differences in the frequency of variants of *Chrysemys picta* "slow" haemoglobin—an example of "temperature anti-adaptation"? *Comp. Biochem. Physiol.*, 18, 627-637.

- MAXWELL, L. G. H. 1968. *Atlas of the Great Barrier Reef*. Elsevier Publish, Amsterdam. (In press.)
- MOORE, H. B. 1958. *Marine Ecology*. Wiley, New York.
- MOURANT, A. E., KOPÉC, A. C., AND DOMANIEWSKA-SABEZAK. 1958. *The ABO Blood Groups: Comprehensive Tables and Maps of World Distribution*. Blackwell, Oxford.
- NAUGHTON, M. A., MESCHIA, G., BATTAGLIA, F. C., HELLEGERS, A., HAGOPIAN, H., AND BARRON, D. H. 1963. Hemoglobin characteristics and the oxygen affinity of the bloods of Dorset sheep. *Quart. J. expr. Physiol.*, *48*, 313-323.
- NICOL, PATRICIA I., AND O'GOWER, A. K. 1967. Haemoglobin variation in *Anadara trapezia* (Deshayes). *Nature*, *216*, 684.
- PETRAS, M. I. 1967. Studies of natural populations of *Mus*. I. Biochemical polymorphisms and their bearing on breeding structure. *Evolution*, *21*, 259-274.
- RICHARDS, F. A. 1957. Oxygen in the ocean. Treatise on marine ecology and paleoecology. Vol. Ecology (Hedgpeth, J. E., Ed.). Geographical Society of America Memoir 67.
- ROGHFORD, D. J. 1960. Bass Strait. *McGraw Hill Encycl. of Sci. and Tech.* Vol. 7, 59.
- WOOLF, B. 1954. On estimating the relation between blood groups and disease. *Ann. Hum. Genet.*, *19*, 251-253.