Heredity (1974), **33** (3), 409-437

NOTES AND COMMENTS

EXPERIMENTAL EVIDENCE FOR NON-VISUAL SELECTION IN *CEPAEA NEMORALIS*

C. R. BANTOCK
Department of Biology and Geology, The Polytechnic of North London, Holloway, London N7 8DB

Received 28.iii.74

Summary

A population-cage experiment carried out on *Cepaea nemoralis* in a valley bottom which experiences temperature inversions has demonstrated powerful non-visual selection. The brown morph survived significantly better than pink five-bandeds and yellow unbandeds which are naturally absent from the valley. It is suggested that climatic factors are responsible.

1. Introduction

On the basis of relationships between morph frequency and latitude, altitude, aspect and topography, several authors have suggested that climate may cause inter-colony differences in *Cepaea nemoralis* (L.) (*e.g.* Cain and Currey, 1963). Some of the conclusions arising from distributional data are supported by direct (Richardson, 1974) and experimental (Lamotte, 1959) evidence of non-visual selection; yellow unbanded individuals have been shown to be more resistant to temperature extremes than pink five-banded ones. On the basis of a correspondence between brown morph frequency and topography on the Marlborough Downs, Cain and Currey (*loc. cit.*) suggested that this morph was favoured in sites likely to accumulate cool night air; results suggesting a similar response to possible microclimate have sometimes been confirmed elsewhere (*e.g.* Cain, 1968).

The Nettlecombe valley in the Brendon Hills, Somerset, is characterised by marked topographic variation, and Ratsey (1974) has shown that temperature inversions between two meteorological stations at different altitudes occur on 60 per cent of all nights, and that ground frosts can occur in any month at the lower site. Variation in the frequency of brown *C. nemoralis* in this valley supports the interpretation given above (Bantock, in prep.). Apart from browns, only pink unbanded and mid-banded individuals are found (Bantock and Noble, 1973); further west yellows and five-bandeds occur additionally. Yellow unbanded is very rare; in a study area of 121 km^2 this morph represents less than 0·3 per cent of over 13,000 individuals collected.

A field experiment carried out near the lower of the meteorological stations has demonstrated powerful non-visual selection. Although the selective agent (or agents) have not been identified, the direction of the selection suggests a climatic interpretation of variation in gene frequency in the area since the brown morph survived significantly better than those morphs which are naturally absent from the valley in which the experiment was carried out.

2. Methods

Six population cages (80 cm × 64 cm × 64 cm) were put out during March 1973. Cages 1-4 and 6 were arranged next to each other on level ground about 10 m from the lower of the meteorological stations. Cage 5 was placed 100 m away on slightly higher ground. The cages consisted of wooden frames completely enclosed with 1 cm wire mesh, and were sunk to a depth of 10 cm into the ground. They were filled to this level with earth which was planted with nettles and grass. The donor snails were taken from a highly polymorphic population 6 km to the north-west of the Nettlecombe valley, from level ground in a low-lying maritime situation. The sampling area consisted of 270 m of continuous rather uniform bank-hedge. There are slight differences in morph frequency between adjacent 30 m sampling sites but most of the morphs used in the experiment are present in each sample; there is no sign of large-scale clines in morph frequency. Migration probably occurs between adjacent sites but presumably not between the ends of the population. Although the total sampling area cannot therefore be considered to be panmictic, it is unlikely that there are major differences in genetic architecture between different parts of the population.

Only adults were used in the experiment; the snails were put out on 30th May 1973, and scored for mortality on 7th September 1973.

3. Results

The data for each cage are given in table 1. The log-likelihood ratio test (Sokal and Rohlf, 1969) has been employed; tests of independence and *a priori* tests of partitions are given in table 2. There are highly significant differences in mortality (M) between the morphs (Forms = F) and the

Table 1

The numbers of each morph living (L) and dead (D) at the end of the experiment

Cage:	1		2		3		4		5		6	
Morph:	*L*	*D*	*L*	*D*	*L*	*D*	*L*	*D*	*L*	*D*	*L*	*D*
B00000	11	78	19	53	11	39	9	23	24	44	7	3
P00300	9	75	13	57	12	58	5	24	11	20	7	3
P12345	6	82	4	65	11	39	2	27	2	17	6	4
Y00300	2	18	2	13	2	10	2	5	4	6	1	2
Y12345	5	14	0	16	0	11	2	5	2	6	3	0
Y00000	0	5	0	4	0	2	0	0	0	0	0	0

Table 2

Tests of independence and a priori *test of partitions*

Hypothesis tested	d.f.	G
S × F × M independence	60	156·192 (P < 0·001)
S × M independence	5	68·588 (P < 0·001)
S × F independence	25	40·22 (P < 0·05)
M × S independence	5	22·524 (P < 0·001)
S × F × M interaction	25	25·058 n.s.

S = Sites (cages); F = Forms (morphs); M = Mortality.

relevant *a posteriori* Simultaneous Test Procedure shows that browns survived better than both pink five-bandeds and yellow unbandeds (table 3). Further inspection shows that mid-bandeds have survived better than five-bandeds; $G = 5{\cdot}074$ ($P < 0{\cdot}05$).

It is clear from table 2 that there are highly significant differences in mortality between the cages (Sites = S) and from table 1 that there are differences in density between the cages. A further *a posteriori* STP shows that cages 1-4 can be considered homogeneous with respect to mortality, and that cages 5 and 6 are significantly different from each other and from cages 1-4. Since cage 6 contained the fewest snails (and cage 1 the most), it is likely that mortality was density dependent. Cage 5 is isolated from the

TABLE 3

A posteriori *simultaneous test procedure* : $M \times F$

B0	Y3	Y5	P3	P5	Y0	
	———	———	11·062	———	———	Subsets of maximally non-significant values of G.
———	———	3·766	———			

others and intermediate in density between cages 3 and 4. The lower mortality in cage 5 could be related to its different situation, but clearly more data are required before firm conclusions relating to differences in selection and mortality between the cages can be reached. There is no sign that selection was density-dependent; the non-significant $S \times F \times M$ interaction (table 2) shows that the degree of association between one pair of variables does not differ for the different levels of the third factor.

4. DISCUSSIONS AND CONCLUSIONS

The analysis shows clearly that selection has occurred. Visual selection by vertebrates is excluded; nor is there any sign that dipterous parasites (Van Emden, 1954) are responsible since none emerged when dead individuals with shells still containing soft parts were incubated at room temperature after the termination of the experiment. Although it is not possible to say that the selection occurred as a result of a particular climatic factor, the results provide the first experimental evidence of non-visual selection favouring the brown morph. Since the experiment was carried out in a valley bottom known to experience temperature inversions the results lend weight to Cain's 1968 interpretation of the distribution of this morph. Since five-bandeds are absent from the valley system and yellow unbanded is very rare in the area as a whole, the fact that these morphs survived least well in the experiment suggests that the possibility of differences in the tolerance of different phenotypes to as yet unidentified factors must be considered in the interpretation of field data. It will be of interest to carry out further experiments along the lines described in this paper.

Acknowledgments.—I am grateful to J. H. Crothers of Leonard Wills Field Centre for the facilities he has provided at Nettlecombe, to M. Ratsey for assistance with the collection of snails, and to Dr P. H. Harvey for assistance with the analysis.

5. References

BANTOCK, C. R., AND NOBLE, K. 1973. Variation with altitude and habitat in *Cepaea hortensis* (Müll). *Linn. Soc. Zool.*, *53*, 237-252.

CAIN, A. J. 1968. Studies in *Cepaea* V. Sand-dune populations of *Cepaea nemoralis*. *Phil. Trans. R. Soc. B.*, *253*, 499-517.

CAIN, A. J., AND CURREY, J. D. 1963. Area effects in *Cepaea*. *Phil. Trans. R. Soc. B.*, *246*, 1-81.

LAMOTTE, M. 1959. Polymorphism of natural populations of *Cepaea nemoralis*. *Cold Spring Harb. Symp. Quant. Biol.*, *24*, 65-84.

RATSEY, S. 1974. The climate at and around Nettlecombe Court, Somerset. *Field Studies*, *3*, 741-762.

RICHARDSON, A. M. M. 1974. Differential climatic selection in natural populations of land snail *Cepaea nemoralis*. *Nature*, *247*, 572-573.

SOKAL, R. R., AND ROHLF, F. J. 1969. *Biometry*. Freeman, San Francisco.

VAN EMDEN, F. I. 1954. *Handbook for the identication of British insects. X. Diptera Cyclorrapha: Tachininidae and Calliphoridae*. Royal Entomological Society of London.

THE CONCENTRATION OF S-PROTEIN IN STIGMAS OF *BRASSICA OLERACEA* PLANTS HOMOZYGOUS AND HETEROZYGOUS FOR A GIVEN S-ALLELE

MARGARET SEDGLEY

Scottish Horticultural Research Institute, Invergowrie, Dundee

Received 1.iv.74

Summary

From the measurement of end-point dilution of stigma extracts of *B. oleracea* tested against S-protein antisera it was found that stigmas homozygous for a particular S-allele contained more specific S-protein than those heterozygous for the same S-allele. There was generally twice as much S-protein in the homozygote as in the heterozygote.

1. Introduction

NASRALLAH AND WALLACE (1967*a*, *b*) described a method whereby antisera were produced in rabbits to stigma extracts of *B. oleracea* genotypes of known S-allele constitution. Some sera contained an antibody thought to be specific for a particular S-allele and the antigen was called the S-protein. To test the association between S-allele and S-protein, plants that had been used to induce serum production were crossed to other S-genotypes and the F_1 and F_2 progenies raised and tested. It was found that the capability of any offspring to stimulate production of the specific S-protein was correlated absolutely with presence of the specific S-allele (Nasrallah, Barber and Wallace, 1969; Nasrallah, Wallace and Savo, 1972). This paper reports the measurement by serological techniques of the relative concentration of S-protein in plants homozygous and heterozygous for S-alleles.

2. Materials and methods

S-allele antisera were produced in rabbits, as described by Sedgley (1974, in press), to stigma extracts of two kale plants, one homozygous for the