"AREA EFFECTS" AND NON-ADAPTIVE VARIATION BETWEEN POPULATIONS OF *CEPÆA* (MOLLUSCA)

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IN a recent study of the polymorphic land snails *Cepæa nemoralis* (L.) and *C. hortensis* (Müll.) living on the chalk downs near Marlborough, Cain and Currey (1963*a*) find significant genetic variation between colonies in similar habitats, as well as resemblances in others from different habitats. Shell colours and banding patterns have elsewhere usually been found to match the vegetation where they occur, which is believed to result from visual selection, for example by thrushes picking out yellow and banded shells in beech woods where they are conspicuous, though these are cryptic on green grass and come to predominate on such backgrounds (Cain and Sheppard, 1954). By contrast, on the chalk downs various different genes approach saturation regardless of habitat over areas of up to a few square miles, with often a very sharp change-over between contiguous areas. These are to be called "area effects", defined by a constancy of morph frequency over large and diverse areas in spite of visual selection.

There are no apparent differences in vegetation, soil, climate, or topography between the different areas, but they and their snail populations are certainly much too large for the genetic variation to be ascribed to the Sewall Wright effect of random errors in gene assortment operating from one generation to the next *at the present time*, and Cain and Currey (1963*b*) conclude that "... the sizes of the areas and of the populations enclosed are so great that the possibility of genetic drift being an important factor can be neglected". They are therefore disposed to look for other "selective forces, certainly not visual", perhaps unrecognised minor climatic differences, to account for the area effects. Other selective agencies could also be suggested: for example, an epidemic disease killing off only certain specific genotypes might have afflicted some areas more than others, as Pettenkofer *et al.* (1962) have proposed to explain similar area effects with the human blood group genes.

However, the interesting observation from archæological sites nearby, that the area effect for brown shells may have existed since neolithic times, argues against such possibilities, for the effects of haphazard disease incidence could hardly have lasted so long, and over this period there have been secular changes in climate which must surely have had a greater effect than any local variation on the Marlborough Downs at the present day. But if non-adaptive chance is to be excluded, the genetic differences between the areas must be adaptive and due ultimately to differential environmental selection.

Before invoking unidentified environmental selective forces, however, one must be sure that random events, past as well as present, really can be neglected. Cain and Currey believe that the fairly extensive changes in agricultural use recorded over the past two centuries are not likely to have reduced the snail population to a few separated nuclei small enough for random assortment to have led to genetic diversification, so that these could have spread out to give the observed area effects on the "Founder Principle" of Mayr (1942), which is a special case of the Sewall Wright effect. This, however, is not long enough: the chalk downs must have been much as they are now, and populated by *Cepaea*, for many thousands of years, but whenever it was that the first colonists arrived they may well have been few and far between, and they and their descendants will not necessarily have constituted a genetically uniform population over the whole area. The crucial question is whether such chance differences could have endured for hundred or thousands of years in the face of uniform environmental selection.

Visible polymorphism in *Cepaea* involves about half a dozen gene loci, some with multiple allelomorphism, and there may be others whose effects are not so easily seen. This is maintained at least in part by heterozygote advantage, so that for example yellow shells, due to a recessive gene allelomorphic with pink, are seldom eliminated entirely even from beech woods, where they often comprise more than 10 per cent. of the population although they are highly conspicuous in this habitat. The balanced polymorphism that is actually achieved in any colony results from the interaction of external selective forces with internal genotypical selection among the snails themselves. But since a lot of different genes are involved, it may well be that more than one combination is able to strike a satisfactorily adaptive balance in the same environment with identical external selection, as Dobzhansky (1957) has found with *Drosophila* in population cages. The balanced polymorphism will then be being maintained by environmental selection working together with internal selective pressures, and it may change in response to changing environmental conditions, though which of several possible balances is actually reached will be determined primarily by the composition of the gene pool upon which external selection has been operating. That could well owe a lot to the chance assortment of genes in a very small foundation stock, which had then extended its range and multiplied its numbers far beyond the possibility of any further random drift.

If two colonies reach different balances of polymorphism in this way and then extend their areas until they meet, any hybrids produced will belong to neither of the two balanced polymorphic systems and are likely to be less viable than their parents. That will therefore form a bar to further interpenetration, and there will be a more or less narrow and stable band along the line of contact, with a steep cline between the phenotype frequencies characteristic of the two colonies.

This is what was found on the Marlborough Downs, where the steepest cline showed an increase in brown shells from 12 per cent. to 93 per cent., with yellows decreasing from 80 per cent. to 0 per cent. and pinks staying constant at 7-8 per cent., over a distance of about 150 metres, and there were other examples of steep clines over a few hundred yards for several different shell banding phenotypes. It would be worth making experimental crosses between snails taken from the ends of these clines, to see if there was any evidence of hybrid inviability or assortative mating. Such a finding would confirm the hypothesis of differences in balanced polymorphism between the areas, and it would not be expected if the genetic variation was due solely to differential environmental selection.

Different systems of balanced polymorphism are unlikely to be selectively identical in all respects, and if one of them enjoys a selective advantage over another with which it is in contact, it will tend to invade the territory of the latter and the cline in morph frequency, marking the line of contact, may be expected to move although it should remain narrow and steep so long as there is no viable introgressive hybridisation between the two balanced systems. In a continuous habitat, with no physical barriers to prevent dispersion, all the weaker populations will in the end be eliminated in this way, provided the selective situation does not change. But if the balance of advantage is shifting from one to another, in response to seasonal or longer-term climatic changes for example, a dynamic equilibrium could be established with the position of the morph frequency cline highly sensitive to environmental change and liable to move backwards and forwards with long-term climatic variation.

On the Marlborough Downs, where the area effects with *Cepæa* are large and long established but rather few in number, those balanced polymorphic systems that have survived in contact with one another are likely to have reached this sort of equilibrium, for any that had not done so would long since have disappeared. The morph frequency clines between different area effects may therefore be sensitive to climatic or other changes in selection pressures, and they may well be moving though possibly too slowly for this to be detected. But although this would show that the different populations were not identical in their reactions with the external environment, it should *not* be taken to indicate that the genetical differences between them, seen in these area effects, must necessarily have resulted from local differences in environmental selection between the areas concerned. That is only one of the possible explanations, and it needs to be proved by showing that there really are some consistent climatic or other environmental differences sufficient to have produced the significant genetical differentiation observed.

If in fact the area effects on the chalk downs are non-adaptive and originate from random assortment in the foundation colonies, they must certainly have come into being a long time ago. Land snails

are known to be very sedentary in undisturbed habitats, and with the rates of dispersion estimated for *C. nemoralis* (Goodhart, 1962) they would take centuries to spread out over such areas. It is not therefore surprising that area effects as striking as these should first have been recognised in a region known to have been undisturbed for thousands of years. For the same reasons they are to be expected also in heathlands and on stabilised seaside sand dunes, but in mixed agricultural districts, where there has been a lot of disturbance, any anciently large area effects are likely to have been broken up with the spread of cultivation. If new balanced polymorphic systems have become established by the Sewall Wright effect in small foundation stocks in different places, they will usually not have been able to spread out over large and diverse areas, as they have done on the downlands, and they will not so easily be recognised for "area effects".

There is of course wide intercolony variation in these mixed districts and, although some of it is known to be adaptive, there are also important genetic differences between colonies in rather similar habitats, which are not so easily explained. For example Cain and Sheppard (1954), in a series of about fifty samples from localities around Oxford described variously as "Hedgerows" and "Rough Herbage", found yellow shells ranging from 18 per cent. to 100 per cent. and effectively unbanded from 0 per cent. to 70 per cent. It is true that these will probably have included a number of different habitats with different selection pressures, but they seem all to have been of much the same general type and it is surprising that there should be quite such wide variation amongst their snails, resulting solely from environmental selection. Even if visual predator selection against certain phenotypes is effective in some habitats, one cannot assume that it will be equally effective everywhere else, especially as it is now seen to be ineffective in overriding the area effects on the chalk downs. So although the inter-colony variation in rough herbage *could* result from predation, that remains to be proved: some of it might equally well represent small-scale area effects and it cannot be taken for granted that these, any more than the larger ones on the chalk downs, are necessarily due to differential environmental selection alone.

Although snail colonies in rough herbage are commonly too large for genetic drift, they often occupy quite small areas that could be filled in a matter of decades rather than centuries. They are therefore well situated for the establishment of different balances of polymorphism in similar environments by internal genotypic selection acting upon gene pools at one time small enough for chance to have had a significant differentiating effect. So again it should not be taken for granted that all of this inter-colony variation must be adaptive, hard though it may be to prove the contrary. There is indeed already some evidence that founder effects are able to produce such small-scale non-adaptive variation in *Cepaea*. An abundant colony of *C. nemoralis* living along two miles of an unusually uniform river bank in the fens near

Cambridge (Goodhart, 1962) shows a range of genetical variation quite comparable with that in Cain and Sheppard's fifty rough herbage colonies around Oxford. There is good reason to believe that this has not resulted from differential environmental selection, but from non-adaptive founder effects when the population had been greatly reduced by flooding five years previously. Further unpublished work shows that this variation is still maintained nine years after the first observations, and there are still steep clines in phenotype frequency over distances of 100 yards or so along the bank, though it is as yet too early to say that these have become stabilised by selection between different systems of balanced polymorphism.

To prove that the Sewall Wright effect, either as continuing genetic drift or once for all in a small foundation stock, has been responsible for inter-colony variation one must show first that the effective breeding populations concerned have been at some time small enough for sampling errors to have been important, and then secondly that the observed variation is *not* due to differential environmental selection. This can be done experimentally in population cages where the environment is strictly controlled, but in nature it is rare to find two separate populations living under perfectly identical conditions, and rarer still to be able to prove that some significant differentiating factor has not been overlooked. Another approach is to study colonies living in broadly similar but ecologically varied habitats. If some inherited characteristic occurs in one colony throughout a variety of habitats, and not in another covering a similar variety, it could be argued that the intra-colony range of ecological diversity is greater than any consistent inter-colony differences, so that the genetical variation cannot have been determined ecologically. Even here however it would be hard to be sure that nothing important had been overlooked.

This seems to be the position with the "area effects" in *Cepæa* on the Marlborough Downs. They do override visual predation, and any other selective differences there may be between the rather dissimilar habitats of open downland turf and beech woods, but one cannot be sure that there are no other differentiating factors, however little indication of them there may be. One thing is nevertheless clear, that although the snail populations are now much too large for genetic drift, they could very well have started with foundation stocks small enough for the Sewall Wright effect to have been important. Furthermore, genetical diversity originating in this way by chance could be maintained indefinitely by internal genotypical selection for different balances of polymorphism in various areas, without any differences in external environmental selective forces. Thus the fact that these populations are now much too large for genetic drift cannot be held to indicate that the genetic variation between areas must necessarily be wholly adaptive and due to unidentified selective differences in the external environments. Until there is some good evidence that effective environmental differences do exist, these "area effects" might as well

be regarded as examples of non-adaptive variation resulting from the Sewall Wright effect in foundation stocks, as good as any that could be expected to be found in natural populations not subject to experimental control.

SUMMARY

Cain and Currey (1963) have described "area effects" in *Cepaea* populations on the chalk downs near Marlborough, where different genes approach saturation over areas of up to a few square miles regardless of habitat and of the effects of visual predation, and often with steep clines in phenotype frequency between adjacent areas. Since the snail populations are now much too large for genetic drift, it has been concluded that this genetic differentiation must be adaptive and due to selective differences in the external environment, though what these may be is not yet known.

There is still however the possibility that chance has been responsible for the origin of this genetic diversity, operating not at the present time, when the numbers involved are certainly much too large, but a long time ago according to Mayr's "Founder Principle", when the colonies were first established. Some of the implications and consequences of this view are discussed. The facts appear to be consistent with the hypothesis that the area effects may have resulted from populations of originally different genetical composition, due to chance assortment in small foundation stocks, having evolved different systems of balanced polymorphism in the various areas when exposed to the same selective forces in the external environment. When the original populations spread out and eventually meet, further interpenetration is prevented by the relative inviability of hybrids between the different balanced systems, so that a cline becomes established along the line of contact between them, and is maintained indefinitely in a state of dynamic equilibrium. It cannot therefore be taken for granted that the genetical differences in the area effects must necessarily result from differential environmental selection: it may have done so but that is not the only possible explanation, and the question should remain open until evidence can be found that there really are consistent environmental differences between the different areas concerned.

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