
A two-stage model for *Cepaea* polymorphism

L. M. Cook

The Manchester Museum, University of Manchester, Manchester M13 9PL, UK

The history of the study of snails in the genus *Cepaea* is briefly outlined. *Cepaea nemoralis* and *C. hortensis* are polymorphic for genetically controlled shell colour and banding, which has been the main interest of the work covered. Random drift, selective predation and climatic selection, both at a macro- and micro-scale, all affect gene frequency. The usual approach to understanding maintenance of the polymorphism, has been to look for centripetal effects on frequency. Possible processes include balance of mutation pressure and drift, heterozygote advantage, relational balance heterosis, frequency-dependent predation, multi-niche selective balance, or some combination of these.

Mutational balance is overlaid by more substantial forces. There is some evidence for heterosis. Predation by birds may protect the polymorphism, and act apostatically to favour distinct morphs. Although not substantiated for *Cepaea*, many studies show that predators behave in the appropriate manner, while shell colour polymorphisms in molluscs occur most commonly in species exposed to visually searching predators. It is not known whether different thermal properties of the shells help to generate equilibria.

Migration between colonies is probably greater than originally thought. The present geographical range has been occupied for less than 5000 generations. Climatic and human modification alter snail habitats relatively rapidly, which in turn changes selection pressures. A simple simulation shows that migration coupled with selection which fluctuates but is not centripetal, may retain polymorphism for sufficiently long to account for the patterns we see today. There may therefore be a two-stage basis to the polymorphism, comprising long-term but weak balancing forces coupled with fluctuating selection which does not necessarily balance but results in very slow elimination. Persistence of genetic variants in this way may provide the conditions for evolution of a balanced genome.

Keywords: polymorphism; *Cepaea nemoralis*; migration; metapopulation

1. INTRODUCTION

In the snail genus *Cepaea* the species *C. sylvatica* and *C. vindobonensis* live in the Alps and the Balkans-to-Caucasus respectively, have a pale-yellow to white shell ground colour and are polymorphic for banding type. *C. nemoralis* and *C. hortensis* are polymorphic for both banding and ground colour. They have broadly overlapping ranges in western Europe, the former extending further south into Italy and the Iberian peninsula and the latter extending further north in Scandinavia and to southern Iceland. They live in a very wide range of habitats, from deciduous woodland to sand dunes, low-lying sunny southern valleys to high altitudes on limestone mountains. They are relatively slow-growing and long-lived, individuals surviving seven or more years and a generation being 2.5–3 years. The first two have a haploid chromosome number of $n=25$, while the second two have $n=22$ (Perrot 1938; Bantock 1972; Patterson & Burch 1978; Page 1978; Gill & Cain 1980). It is reasonable to suppose that an ancestor of *C. nemoralis* and of *C. hortensis* had both the smaller chromosome number and the ground colour polymorphism, and existed in south-western Europe before speciation and northward spread. Fossil *Cepaea* species are known from the Oligocene onwards. More than 40 species have been named, most of them fossils from the Miocene to Pleistocene of Europe, some from Turkey (Berz & Jooss 1927; Wenz 1960;

Richardson 1980; Zilch 1986; Grossu & Badarau 1990). Zilch (1986) includes *C. litturata* (Pfeiffer) as an extant member of the genus living in southern Spain and north Africa. It is a polymorphic species with $n=22$ (Perrot 1938), also referred to *Pseudotachea coquandi* (Mar.) (Sacchi 1965). *C. nemoralis* is recorded from the Miocene, and *C. hortensis* from the Pliocene (Taylor 1914; Thomaz *et al.* 1996; R. C. Preece, personal communication). Although bands are discernible on fossils it is not possible to tell what colours the shells were. We may conjecture that the split between the species with larger and smaller chromosome numbers occurred well before the Pleistocene, and that they expanded and contracted in range with the principal changes in climate which subsequently took place. The present northern distribution of *C. nemoralis* and *C. hortensis* must have been established sometime after the last advance of ice about 10 500 years before present (BP); they were evidently common in Britain by the warm period of 6000 yr BP (Currey & Cain 1968; Cain 1971). Species in many groups of plants and animals showed rapid northward movement at that time (Hewitt 1996).

In volume 1 of the *Zoological Record*, covering 1864 and earlier, there were no citations of the species now included in the genus *Cepaea* (at that time also referred to as *Helix*). From then until 1990 (vol. 127) more than 750 references appeared, probably an underestimate of the total published. The pattern of entries reflects changes of

interest over the period. Apart from interest in its polymorphism, *Cepaea* has also become a model for research on physiology and neurochemistry (for examples, see Hryk *et al.* 1992; Kavaliers 1992; Dyakonova *et al.* 1995a,b). The early work addressed the following topics.

1. Varietal and geographic variation. A total of three main shell colours were recognized at an early date, brown, pink and yellow, each of these categories being divisible into light or dark shades. Sometimes the shell is bandless, but frequently there is a pattern of bands, usually dark brown, running round it. In volume 3 of the *Zoological Record* (1866), E. von Martens reviewed the work of G. von Martens and others which showed that a banded individual potentially carries five bands at fixed positions on the shell. Any band can, in principle, be absent and any contiguous pair may be fused to form a broader stripe. Realization of the existence of this scheme and of the variation in ground colour led to an interest in mapping the geographical distribution of the patterns and, initially through interest in the occurrence of rarities, in recording morph frequencies. *C. nemoralis* and *C. hortensis* have homologous polymorphisms for shell ground colour and banding; they often coexist in the same habitats with different morph frequencies.

Both species have been introduced to north America, and from an early stage there has been interest in the parallels between the colonies in the new localities and those of Europe. Many publications, that in themselves are no more than unconnected records of frequencies, were brought together in later surveys, such as those of the Schilders (1957), and the information was passed on verbally from one specialist to another.

2. Genetics. Papers indicating that banding patterns segregate were published before the end of the century (Baudelot 1868; Arndt 1878). The genetics were established by Lang in a series of papers from 1904–1912 (Lang 1904, 1912; Darbishire 1905). The species became material for the developing interest in genetic variability. Shell colour was shown to be determined by an allelic series, darker colours being dominant to paler ones, unbanded dominant to banded. There is no incomplete dominance that would allow genotypes to be identified in the field, although some pink–yellow heterozygotes have a yellow apex which permits identification in bred material (Cain *et al.* 1960). Colour and banding alleles tend to segregate together. Fisher & Diver (1934) drew attention to an apparent case of high recombination between the loci, and some other data suggest recombination of about 1% (Cook & King 1966), but linkage is close and strong disequilibrium is common in the field. The genetics have been thoroughly investigated by Lamotte (1951) and Cain *et al.* (1960, 1968) and reviewed by Murray (1975). Colour, presence or absence of bands, and some loci producing transparent, spotted or other variant band types form a close linkage group of five loci. The unbanded phenotype is epistatic to the alleles at the band modifying loci, as the latter are only expressed in the five-banded homozygote. Two loci which reduce a genetically five-banded phenotype to one with three or

a single band are unlinked to this group or to each other, and at least four other loci are involved.

A polymorphic population is defined conventionally as one where the commonest type (allele if the system is established genetically, or phenotype if not) has a frequency of not more than 99%. In *C. nemoralis* the commonest morph frequency is rarely more than 90%. Fewer than 20 of 3000 British colonies reviewed by Jones and co-workers (1977) were monomorphic, and many segregated for up to six loci. The species is a natural choice for the study of factors that maintain polymorphism and influence frequencies.

3. Sympatric species. One important question settled at an early stage was whether *C. nemoralis* and *C. hortensis* were indeed two species. The first normally has a dark-brown lip to the shell and the latter a white lip, but both are polymorphic for this character in some places. *C. nemoralis* has a more southerly distribution than *C. hortensis*, but where they overlap they are often sympatric. The question to be answered was whether they are always distinct, or are a complex in which there is introgression in some places (see, for example, Brockmeier 1888), but sexual isolation in others. The work of Lang (1908) showed that rare hybrids could be obtained which had genital and dart morphology distinguishing them from the parental species. Other aspects of the anatomy also differ (Aubertin 1927). Lamotte (1951) reviewed the evidence, which together with many observations of distinctness in the field (see, for example, Guerricci *et al.* 1988), confirmed that two species are undoubtedly involved.

Thomaz and co-workers (1996) show mtDNA sequences in the species to be different. The possibility and nature of interaction and competition between the two has been examined (see, for example, Arthur 1978, 1982; Tilling 1985a; Cowie & Jones 1987; Mazon *et al.* 1989; Arthur *et al.* 1993), as have correspondence in variation of homologous isoenzyme systems (Ochman *et al.* 1987; Guiller & Madec 1991) and chiasma frequencies (Price & McBride 1981).

2. MAINTENANCE OF POLYMORPHISM

(a) *Background*

Early general statements about reasons for the polymorphism in *Cepaea* gave neutrality of the alleles (for examples, see Haldane 1932; Diver 1940; Huxley 1942). There are several reasons why this position lost currency. As the population genetics of this system developed reviews were undertaken that analysed the possibilities, especially important being by Lamotte (1951), Cain & Sheppard (1954), Jones *et al.* (1977), Clarke *et al.* (1978), Cain (1983) and Goodhart (1987). The way attitudes to the visible polymorphism have developed can be seen by highlighting some of their themes.

Lamotte (1951) made a comprehensive survey of what was known of the genus. He demonstrated the basis of the genetics, and presented a large body of survey data from several regions of France. A survey of mating individuals indicated that they associate at random with respect to colour and pattern. In the course of these studies he noted the characteristic frequency distributions which

Table 1. *Associations between polymorphism in pulmonate molluscs and various environmental, geographical and biological factors* (Modified from list in Clarke *et al.* (1978).)

pulmonate molluscs are less likely to be polymorphic for shell colour and pattern:

- (1) in species living on the ground than in those which periodically or permanently climb above it,
- (2) in species of dense woodland than in those of more open habitats,
- (3) if main predators hunt by smell rather than by sight,
- (4) in very small or large species than in those of intermediate size,
- (5) if the average density is low rather than high,
- (6) if the species inbreeds rather than outbreeds,
- (7) in colder, dryer environments than warmer, wetter ones,
- (8) in species of continental land masses than those of archipelagos,
- (9) in aquatic than terrestrial habitats.

were obtained. It appeared that climatic or ecological conditions or predation sometimes favoured particular morphs, but in an erratic way from one site to another. Selection did not clearly maintain polymorphism, whereas small populations showed more variation in morph frequency than large ones (Lamotte 1952). Given this information, Lamotte sought a centripetal process to provide a stable equilibrium. Applying a theoretical model of Sewall Wright, he proposed a balance of forward and reverse mutation operating in populations of limited size. From the modal values of morph frequency, the shape of the frequency distributions and estimates of population size (of about 1000) he derived estimates of mutation rates of the order of 10^{-4} . Thus, the polymorphism could be present because of mutation, while drift and selection operated to cause differences from site to site and between regions.

These mutation rates are much higher than usually observed. The discrepancy would be reduced if the population size estimate is too low. If it went up by an order of magnitude, mutation rate would correspondingly come down, although a given level of selection would then have more influence on frequency. As Lamotte noted, mutation is indistinguishable from migration from colonies with different frequencies. What was estimated as a high mutation rate could result from migration at quite a low level, so long as diversity between colonies was maintained.

Lamotte's (1951) conjecture was criticized by Cain & Sheppard (1954), because of the high mutation rate, because Lamotte chose to examine gene loci (unbanded against banded), rather than the composite phenotypes resulting from linkage disequilibrium (e.g. pink unbanded against yellow banded) and for several other reasons. In their own work (Cain & Sheppard 1950), they had already demonstrated an association of dark phenotypes with dark habitats and pale with paler and more diverse ones and shown that song thrushes (*Turdus philomelos*) discover the more conspicuous individuals against a given background. A large data set illustrating the background association is provided by Jones (1973), from the work of Sheppard (1951), Cain & Sheppard (1954), Clarke (1962*b*), Cain & Currey (1963*a*), Currey *et al.* (1964) and Carter (1968). In addition to song thrushes, *Cepaea* are known to be eaten by a variety of birds (references in Cain & Sheppard 1954; Kohler 1990), and by glow worms, rodents and rabbits (references in Cain 1983), which vary in the way they find and attack the snails.

Where present, the song thrush is the species most likely to exert consistent selection owing to the large numbers eaten during the birds' breeding season. The background matching and strong disequilibria implied appreciable selection, certainly more than 1% per generation. A number of types of evidence pointed to non-visual selection as well, both due to factors such as climate and to constraints of organization such as the one which prevents dark-brown individuals from being banded.

Boettger (1931) had proposed that selection for different morphs in different places, coupled with gene flow, could maintain polymorphism, but Cain & Sheppard (1954) agreed with Lamotte (1951) that there was no sign of such a balance. Their position on the question of balance was that polymorphism is the result of some type of selection which maintains variability independent of locality. If so, there are two possible types. Selection may favour alleles because they are rare, and some bird predation studies indicated that birds might act in the appropriate way. Alternatively, there may be single-locus heterozygote advantage or heterosis within multi-locus linkage groups held together by a mechanism such as chromosome inversion. The *Cepaea* example agreed with several others discussed by R. A. Fisher in which super-genes remained as stable polymorphisms, often with a recessive morph occurring at a high frequency.

Clarke *et al.* (1978) considered genetic variation in pulmonate molluscs as a whole, and continuous as well as discontinuous variation. They suggest that the widespread incidence of polymorphisms for shell coloration shows the associations listed in table 1. Some of the associations noted have no apparent explanation as they stand, for example, that of polymorphism with archipelagos, and some have notable exceptions, for example, *Liguus fasciatus* inbreeds but is polymorphic (Hillis 1989; Hillis *et al.* 1991). Nevertheless, they are important indicators of a general proposition, that polymorphic species are probably intermediate in size, apparent on foliage or other surfaces and subject to visual predation. It should be noted that some of the polymorphisms, for example, in *Helix aspersa* or *Arianta arbustorum*, produce less striking differences between the morphs than others, such as *C. nemoralis*.

Selection acting on discontinuous morphs was discussed by Clarke *et al.* (1978) under three headings: (i) experiments and observations on selective agents; (ii) associations of phenotype frequencies with environmental factors; and (iii) observations of temporal changes in

frequency. They reviewed studies on all these, subjecting them to meticulous scrutiny to detect signs of shortcomings of design or experimental procedure. Although many examples were shown to have weaknesses, the reader, uncowed by the critical process, nevertheless comes from their review feeling that a degree of consistency in results has been achieved. Morph frequencies may be related to backgrounds (Cain & Sheppard 1950), thrushes feed selectively (see, for example, Sheppard 1951), there are correlations of morph frequencies with local topographical features (Cain 1968; Arnold 1969) and with larger-scale variation in climate (Jones 1973). Populations subject to environmental stress may suffer selective mortality (Richardson 1974). Changes in morph frequency have been observed over the short-term, which indicate selection of 3–5% per generation (Murray & Clarke 1978), and over some thousands of years (Currey & Cain 1968).

As to maintenance of polymorphism, Clarke *et al.* (1978) listed four possible processes: (i) selective neutrality; (ii) balance of selection and migration; (iii) heterozygote advantage; and (iv) frequency-dependent selection. As discussed, the first of these cannot be the important stabilizing process if there is sufficient selection to overcome the centripetal tendency owing to mutation. Apart from its advocacy for *Cepaea* by Boettger (1931), the second had received little attention. The third includes multi-locus heterosis as well as heterozygote advantage, and was advocated by Cain & Sheppard (1954). As to its importance, no more could be said one way or the other.

Clarke *et al.* (1978) came down very heavily in favour of frequency-dependent predation favouring rare forms as the factor responsible for polymorphism in *Cepaea*, citing many examples of experimental studies with song thrushes and artificial prey or with other species of predator and prey, which show that predators behave in the appropriate way. Further examples are discussed by Allen (1988), Allen *et al.* (1988) and Greenwood (1984), and more recently by Tucker (1991). The selection was termed apostatic by Clarke (1962*a*), because one of the consequences is that distinct rare forms are less likely to be attacked than ones which converge in appearance on the common form, so that the selection favours distinctness between morphs.

Given these observations, it is very reasonable to suppose that *Turdus philomelos* and possibly other bird predators sometimes act in such a way as to maintain polymorphism in *Cepaea*, and perhaps account for the distinctness of the phenotypes, but there is no direct evidence that they do so. Clarke (1962*b*) used a negative association of morph frequencies in mixed colonies of *C. nemoralis* and *C. hortensis* as evidence for frequency-dependent predation, but other interpretations of this result are possible (cf. Carter 1967; Clarke 1969). Thrushes are not present throughout the distribution of *Cepaea*, and vary greatly in density where they are present. Furthermore, as Ayala & Campbell (1974) and Clarke *et al.* (1978) noted, the response of predators may be labile, depending on ecological conditions. Behaviour that protects rare forms when the prey is at low density may act against them if the prey becomes common (Greenwood 1969; Cook & Miller 1977; Horsley *et al.* 1979), and the response may be influenced by the nature

of the background (Cook & Kenyon 1991). These features reduce the likelihood that frequency-dependent selection could be the universal generator of equilibrium.

Jones *et al.* (1977) restricted their attention to polymorphism in the genus *Cepaea*. They made a thorough review of evidence of association between morph frequencies and climate. In *C. vindobonensis* individuals with fully pigmented bands heat faster in the sun than those with reduced pigmentation (Jones 1973). In some places there is an association of a reduced-banded morph with warm microclimate (Jones 1974) whereas in other parts of its range morph frequency is unconnected with any identifiable component of the environment (Jones 1975). Like

C. nemoralis, morph frequency in this species sometimes shows association with ecology and is presumably subject to selection, but sometimes other unexplained factors operate to produce genetic variability.

Likewise, *C. nemoralis* morphs are affected differently by solar radiation (Heath (1975), and more recently, Steigen (1979); Jones 1982; Tilling 1983, 1985*b*; Chang 1991*a*). Behavioural changes occur as a result (Jarenovic & Rollo 1979; Bergstrom & Whelan 1988). Kavaliers (1992) found that differences between morphs in responsiveness to temperature arise from differences in opioid modulation, therefore demonstrating a chemical basis for the behaviour patterns. Broad associations of morph frequencies and climate also exist. Data from over one million individuals in 10 000 colonies show yellows to be commoner in the hotter parts of the range, whatever their precise geographical location (Jones *et al.* 1977). An exception is described by Vicario *et al.* (1988) in the north of Spain, where the Atlantic region has a high frequency of yellows, whereas the more continental inland region has a lower frequency; the authors discuss reasons why this particular association should occur there. Dark-brown individuals are northern in distribution. They are particularly associated with complex sand dunes with large enclosed hollows, and in the past were most common in periods of colder climate (Cain 1968, 1971). Associations of this type, which have been extended to extreme habitats (Arnold 1968, 1969; Ramos 1985; Mazon *et al.* 1987), to local changes with changing microclimate (Cameron 1992) and to the study of body colour (Cowie & Jones 1985), show that climate may influence morph frequency but do not indicate how it might generate stable equilibria. The review of Jones *et al.* (1977) continued by weighing up the relative importance of the factors outlined here, without clearly favouring one over the others as the cause of polymorphism. Rather, the authors suggested that interaction of several types of selection produces the observed result.

Like Clarke and co-workers, Cain (1983) discussed a range of terrestrial molluscan species; like those authors, and partly in response to their review, he devoted space to the question of experimental design and interpretation. With respect to *Cepaea* he emphasized the massive linkage disequilibria to be found in wild populations, and noted that where we have evidence of the genetic control of visible polymorphisms in other unrelated species there is usually a super-gene present. Super-genes may be connected with chromosomal inversions, and a number of studies have been done on *C. nemoralis* and *C. hortensis* to examine chromosome morphology and chiasma frequencies

(Bantock 1972; Price 1975; Price & McBride 1981). Although there is much variation in chiasma frequency between localities, chiasma frequency on the largest chromosome is low and localized in position. Inversions have not been detected, but some other mechanism may operate to limit crossing over. With regard to climatic selection, selection for crypsis and apostatic selection, Cain (1983) provided a valuable examination of the evidence but no new view of the centripetal mechanism.

(b) *Area effects*

One topic which runs through the studies, is the question of area effects. Subsequent to the demonstration of a good correspondence between habitat and morph frequencies in one part of England, Cain & Currey (1963*a,b*) explored new territory and found a different situation. Colonies within one area had a stabilized frequency of one of the morphs, without apparent reference to the nature of the habitat. The frequency changed rapidly to a different one at a border which was not a detectable habitat boundary. Another locus would exhibit a similar pattern, but the borders did not quite coincide with those of the first. Illustrations are given by Cain & Currey (1963*a,b*), Carter (1968) and Jones (1973). No evidence of selection by visual predators was evident. Like the term 'meiotic drive', the term 'area effect' was introduced to draw attention to an unexplained phenomenon or phenomena. The reasons for different area effects were not necessarily the same, but they needed to be understood to be able to understand the polymorphism as a whole. Cain & Currey favoured the view that some underlying but unseen selective force operated to give rise to them. Goodhart (1963) disagreed, suggesting that they reflected the frequencies present accidentally in the few immigrants which had founded colonies in previously unoccupied territory. The borders occurred where their descendants met, and existed because the occupants of different areas had developed differently coadapted genotypes consequent on the small numbers of founders. The idea was developed by Wright (1965, 1978) in relation to his shifting-balance theory of evolution. Clarke (1966) showed that without coadaptation to the extent that Goodhart's model implied, selection on a cline in frequency could cause it to steepen, and may result in non-coincidence at different loci. These ideas have been elegantly reviewed by Gould & Woodruff (1990). They concluded that historical contingencies explain the evidence better than selectionist interpretations, and drew a parallel with their data on the Caribbean genus *Cerion*.

The proposals of Goodhart, Wright and Clarke are based on gene interaction, which would involve the loci controlling the morphs, as well as others. It fits well with a high level of linkage disequilibrium, which in turn would be facilitated by the presence of chromosomal processes limiting chiasma formation. So far as the data go, however, snails from populations in different areas, or even from different regions, suffer no hybrid loss of fitness (Johnson *et al.* 1984), although they sometimes do in the helicid *Arianta arbustorum* (Baur & Baur 1992).

These explanations of area effects include the notion of history of the population. This has been taken further by Cameron and his colleagues (Cameron *et al.* 1980*a,b*; Cameron & Dillon 1984; Cameron & Pannett 1985).

They found a marked historical component in the faunal composition of hedgerow snail communities, as well as effects of soil acidity and climate. Hedges originating near woodland have richer faunas than those in open fields. Up to 100 years are required for the fauna to become diverse (Cameron 1980*b*). Where the habitat has been established for centuries, *Cepaea* morph frequencies vary with habitat, suggesting selection for crypsis. A century or more is necessary, however, for morph frequencies in woodlands to show a good background match. Where habitat is less stable, and subject to changes in management, area effects are likely to occur. Patterns such as those studied by Cain & Currey occur on down land which was at one time heavily grazed and has since grown a lush cover. Although selection may eventually stabilize the frequencies to levels appropriate for the conditions, so that a match with the habitat becomes apparent, what we see may still reflect the gene frequencies founder individuals brought with them.

Goodhart (1962) studied the distribution of morphs along a river bank subject to flooding, where morph frequencies showed haphazard changes, and concluded that the patterns now observed were caused by the random assortment of phenotypes in survivors of floods. He extended this type of explanation (Goodhart 1963) by arguing for coadaptation to establish the sharp boundaries in area effects. Later (1987) he proposed that most land snails are probably genetically heterogeneous as a result of balancing selection within coadapted gene complexes. If the genes involved have visible effects which are deleterious, their expression is suppressed by selection of appropriate modifying genes. Suppression occurs in some other helicids (he cites *H. aspersa* and *A. arbustorum*), which are polymorphic but with morphs less distinct in appearance than those of *Cepaea*. On the other hand, if the colours and patterns are essentially non-adaptive, they simply indicate the existence of the underlying polymorphisms. The morphs are tags, signalling the presence of heterotic chromosomes, not systems for which polymorphism-generating factors have to be sought. In his view, *C. nemoralis* and *C. hortensis* belong to the tag category.

(c) *Molecular variation*

When methods to investigate isozyme variation in populations became available *C. nemoralis* was a natural candidate for study. Some of the detected esterases are food-induced (Oxford 1973, 1978), so that care is needed in interpreting variation. Johnson (1979) confirmed the genetics of six loci and demonstrated that there is considerable geographical variation across England and Wales. Brussard (1975) examined a range of colonies in the USA, concluding that some originated from northern Europe, whereas those in Virginia were of Italian origin. The question of place of origin has also been addressed by using mtDNA (Stine 1989). The degree of variability at enzyme loci which has come to light in European surveys (Johnson 1976; Jones *et al.* 1980; Caugant *et al.* 1982; Ochman *et al.* 1983, 1987; Valdez *et al.* 1988; Guiller & Madec 1991, 1993; Vicario *et al.* 1989), however, limits the use of enzyme data in this way. Although there are large-scale clines in allele frequencies (Ochman *et al.* 1987; Guiller & Madec 1991) few are correlated with the

environment. Associations of frequency and habitat occur at a smaller geographical scale than the surveys already mentioned, but are not consistent (Wilson 1996). Enzymes and visible morphs tend to vary independently; isozymes do not map onto visible area effects (Jones *et al.* 1980). In the Pyrenees, where large surveys have been done, there are geographical subdivisions characterized by associations of isozyme alleles but they do not coincide with obvious barriers to migration (Ochman *et al.* 1983). The simplest explanation of these results is that the loci are subject to random drift. Patterns of concordance of allele frequencies (molecular area effects) could then be the result of periodic drastic reduction in numbers followed by expansion of population size and geographical range (Ochman *et al.* 1983; Selander & Ochman 1983). Results from isozyme studies therefore raise questions about selection, drift and geographical differentiation such as those relating to the visible morphs.

Sequencing of the mitochondrial genome of *C. nemoralis* (Terrett *et al.* 1996) has provided another means to study genetic variation. The mtDNA composition shows similarities to that of the clausiliid pulmonate *Albinaria* (Lecanidou *et al.* 1994; Hatzoglou *et al.* 1995), but there are large differences to other molluscs which have been sequenced, and between these and other metazoa. Thomaz and co-workers (1996) examined variation between geographically distant populations of *C. nemoralis* and found very high levels of polymorphism and of geographical divergence. They conclude that small populations have persisted for long periods with limited migration between them.

3. COMMENTS AND OBSERVATIONS

In searching for a centripetal factor or combination of factors for *C. nemoralis*, Jones *et al.* (1977) listed eight forces known to operate. The climate of the region in which a population is situated (factor 1) may affect the average gene frequency, while the local topography (factor 2), e.g. exposed hillside against the valley bottom, also plays a part. Predators (factor 3) selectively remove the most conspicuous morphs, so that the population becomes relatively cryptic. By frequency-dependent searching (factor 4), they tend to protect the polymorphism, while the way they do so is influenced by population density (factor 5) and presence or absence of *C. hortensis* (factor 6). Migration from an adjacent area subject to different selection (factor 7) will modify frequency, and historical accident may create a particular linkage disequilibrium (factor 8), affecting the way the population responds to selection. Is this a problem with too many solutions, as the title of their paper suggests? Given the range of factors which undoubtedly operate, it is more pertinent to ask, is there a standard model, or consensus view, that accounts for the phenomena observed?

All those who have studied the polymorphism in *C. nemoralis* in any detail would agree that strong selection sometimes acts on the morphs. They differ in their assessment of the commonness of such selective events, but so long as appreciable selection occurs it is unlikely that polymorphism could be protected by mutation operating at the normal rates. Goodhart (1987) argued that the

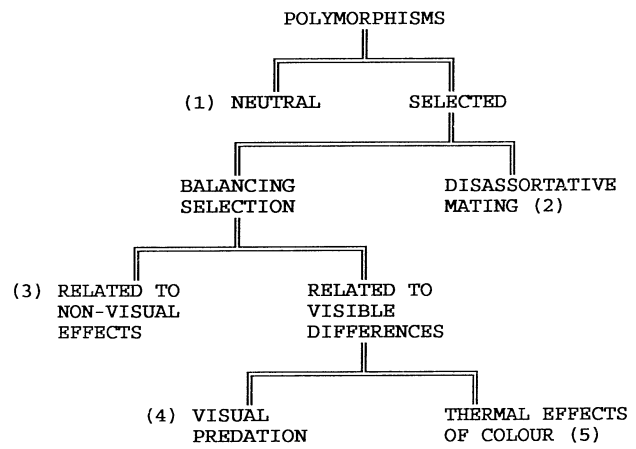


Figure 1. Factors which may maintain molluscan shell colour and pattern polymorphisms. The neutral option (factor 1), where the centripetal process would be a balance of mutation from one morph to another, is possible only if selection is negligible. There is no evidence for disassortative mating (factor 2). Heterozygote advantage or heterosis which does not involve direct selection in visible properties of the shell (factor 3) may occur, but the associations of apparency with polymorphism over a broad range of molluscs, and in *Cepaea* the direct evidence of visual predation and differences of response of morphs to thermal and climatic conditions, show that visible differences are also selected. If predation is frequency-dependent, the factor (4) can be centripetal in effect. For selection via thermal differences (factor 5) to maintain polymorphism requires multi-niche selection. Any of these processes may interact, and all could operate within a single habitat. Balance of selection and migration between habitats is not included in the diagram.

morphs in *Cepaea* were effectively neutral, and that in species such as *Helix aspersa* and *Arianta arbustorum* a cryptic appearance has evolved to cover up the polymorphism and so remove it from any influence of selection. It is difficult to accept this contention, both because *Cepaea* so frequently appears to be subject to morph selection and because the effect of morph difference on behaviour is as strong in *Arianta*, which to Goodhart is cryptic (Parkin 1971, 1972; Abdel-Rehim 1983; Abdel-Rehim *et al.* 1985), as it is in *Cepaea* (Jones 1982; Tilling 1986; and others already mentioned).

Figure 1 gives a tree of possible factors which may be involved in maintaining the polymorphism. On the non-neutral paths, disassortative mating appears to be ruled out. Unless further options are available which are not listed on the figure, there remain polymorphisms attributable to factors unrelated to colour and pattern (heterosis), or some sort of balance based on visible properties.

(a) *Heterosis*

In their different ways, the arguments of Cain & Sheppard (1954) and of Goodhart (1987) are that heterozygote advantage or heterosis supplies the necessary stable equilibrium. Although such arguments tend to be dismissed in the modern literature, this is not because they have been shown to be untrue, rather that they have not been confirmed, and are presently of peripheral interest to the study of molecular variability. Systems such as the inversions studied by Dobzhansky and his associates in *Drosophila pseudoobscura* or the mimetic polymorphisms in

papilionid butterflies studied by Clarke and Sheppard, undoubtedly demonstrate the evolution of heterotic super-genes and thus illustrate an important class of evolutionary event. It is entirely possible that similar systems exist in helicid snails. As Bantock (1972) noted, the restricted distribution of chiasmata along chromosomes, which he observed, would permit the evolution of large permanent linkage groups, thus satisfying one of the conditions for the development of super-genes. A large fraction of helicid species are polymorphic, whether for distinct visible characters or not. Polymorphic species not already mentioned, on which surveys or genetic studies of shell characters have been done and environmental correlates examined are *Theba pisana* (Heller 1981; Johnson 1981; Cain 1984; Cowie 1984; Heller & Gadot 1984; Hazel & Johnson 1990), *Pseudotachea splendida* (Sacchi 1956, 1965), *Xeropicta vestalis* (Heller & Volokita 1981; Heller & Gadot 1987), *Cochlicella acuta* and *Ceriuella virgata* (Lewis 1977), *Helicella candicans* (Honěk 1993), *Trichia striolata* (Jones *et al.* 1974), *Trichia hispida* (Shelton 1984) and *Helix aspersa* (Albuquerque de Matos & Serra 1984a; Lazaridou-Dimitriadou *et al.* 1994; Mulvey *et al.* 1996).

The evolutionary persistence of a heterotic system could be of a very long duration, longer, in fact, than the life of a species. This would explain the presence of shell pattern polymorphisms in many helicid species, which differ in their distinctness and apparent relevance to habitat variation. The proposition cannot be dismissed on grounds of logic or of generalization from other groups of organisms; it has to be tested in the species concerned.

The complement of heterosis is inbreeding depression. Although some snails, such as *Rumina decollata* (Subulinidae) (Selander & Kaufman 1973) or *Liguus fasciatus* (Bulimulidae) (Hillis 1989) undergo extensive self-fertilization, this would not appear to be true of the helicids, which are effectively obligate outbreeders. Chen (1993) found that *Arianta arbustorum* was able to self but confirmed that it normally does not do so. He reported a marked loss of fitness in sib matings compared with matings between unrelated individuals, and concluded that cross-fertilization is maintained by strong inbreeding depression. Albuquerque de Matos & Serra (1984b) found that in *Helix aspersa* sterility increased and fertility dropped over four generations of inbreeding. The effect was so marked in their experiments that they proposed the existence of a more drastic genetic process than simply an increase in homozygosity. *Cepaea* species also appear to engage in obligate outcrossing. When reviewing evidence of self-fertilization Diver and co-workers (1925) found only one possible instance in *C. nemoralis* and one in *C. hortensis*. Like these other helicid species, *Cepaea* may therefore experience inbreeding depression.

The conventional means to measure heterosis would now be to examine how variance in some fitness character changes with multi-locus heterozygosity (see, for example, Zouros & Foltz 1987; Koehn *et al.* 1988), but no experiments of the scale required have yet been made. As far as I know, the only attempt to measure heterosis in *C. nemoralis* is that of Cook & Gao (1996). In *Cepaea*, shell size and shape affect fitness (Wolda 1970). Shell dimensions are to some extent heritable (Cook 1967) and there is reason to believe that they may be subject to selection acting on the adult (Cook & O'Donald 1971).

An alternative approach is therefore to compare variance in individuals carrying dominant shell colour or pattern genes with those that are homozygous for the recessive, as the dominant group includes heterozygotes for the loci concerned and perhaps for sections of chromosome in which these loci are situated. This method has also been considered by C. B. Goodhart (personal communication). The result obtained by Cook & Gao (1996) was a modest tendency for the dominant phenotype to show a lower variance of shell dimensions than the recessive one ($p < 0.05$). The jury is still out on the question of heterosis, however, and it remains possible that persistence of visible polymorphisms is to a greater or lesser extent owing to heterosis of chromosomes which include the colour and banding loci.

(b) Visual selection and polymorphism

Being approximately round, snails may be relatively poorly protected against vertebrate predators, which are attuned to detect circular objects. This argument is supported by the many examples of deimatic behaviour across the animal kingdom involving eyespot patterns, and experimental manipulations of them (see, for example, Wickler 1968; Edmunds 1974). Rather than being closely cryptic, *Cepaea* may therefore tend to resemble distinctive features in a heterogeneous environment (Cain *et al.* 1960; Cook 1986). Various studies mentioned here indicate that visual predation operates, and that predators tend to select their prey in a frequency-dependent manner. Polymorphic pulmonates appear to be associated with the ecological, geographical and behavioural conditions listed in table 1. As arranged here, the first five conditions suggest that polymorphic species are on view and exposed to visually hunting predators. Cain (1977, 1988) gives data showing that in European helicids and in marine bivalves, apparent, exposed and colourful species are more likely to be polymorphic than hidden ones, which are unicoloured dark or white. These results are summarized in table 2a,b. Species in the prosobranch genus *Littoraria* tend to be confined either to the trunk and roots or the leaves of mangrove trees. Table 2c shows numbers of polymorphic species from these two niches in the Indo-Pacific. These associations suggest strongly that polymorphism in molluscs is related to being visible against mixed backgrounds, where individuals are likely to be exposed to predation by sight-hunting predators and, in the case of terrestrial ones, to heating and cooling which acts differently on different-coloured shells. These factors directly affect visible attributes of the shells. As the association is with polymorphism, rather than with brightness as such, the conditions evidently tend to protect polymorphisms. Polymorphism in *C. nemoralis* is therefore likely to matter because of the way the shells appear, and to be protected (to an unresolved extent) by frequency-dependent predation.

(c) Population structure

Lamotte (1951) found that colonies contained 1000–2000 adults as a rule, sometimes as many as 20 000. Subsequent studies have confirmed a picture of somewhere from a handful to a few thousand individuals living in close contiguity, usually well separated from the next

Table 2. *Associations of apparency and polymorphism with behaviour or habitat in a variety of molluscs*

(a) European Helicidae according to favoured behaviour or habitat and general coloration of shell. Last row shows number polymorphic among those listed above it. From Cain (1977), who gives details of the categories used.

	more or		general	exposed	very
	nocturnal or shady	less nocturnal	habitat		exposed
dark	9	5	0	0	0
medium	8	10	7	4	0
light	0	1	2	10	17
white	0	0	0	1	3
polymorphic	0	0	8	10	14

(b) Marine bivalves according to probable degree of visibility to predators and degree of colour and patterning. From Cain (1988).

	well-exposed to predators	partly hidden	hidden
highly coloured and variable	10	11	0
well-coloured	12	20	1
poorly coloured	7	26	12
white	4	17	44

(c) Association of polymorphism with substrate in species of *Littoraria* living on different parts of mangrove trees in the Indo-Pacific region. Data from Reid (1986).

	trunk	leaf
monomorphic and variable	6	3
polymorphic	0	8

such group. Indirect observations give a similar impression. For example, Cain and co-workers (1990) studied six contiguous areas each containing 200–300 individuals, with about 5% movement per year between them. Reviewing the available data, Greenwood (1975) concluded the effective population number was about half the actual neighbourhood number, while neighbourhood numbers are likely to be between 400 and 13 000, with a skewed frequency distribution and a mode of about 6000.

Besides direct examination of numbers, another approach to the question of N_e is to measure the migration of marked snails. At one of his study sites, Lamotte (1951) found an average movement of about 10 m in two years, while at another there was a displacement standard deviation of 9.4 m yr^{-1} . Goodhart (1962) estimated that for a linear colony of *Cepaea nemoralis* there is a displacement standard deviation of about 5.5 m yr^{-1} . Individuals have a strong inclination to stay put.

This picture may not tell the whole story, however. Greenwood (1975), referring to Kimura & Weiss (1964), noted that even small amounts of migration can greatly increase the effective population size, and that long-distance dispersal may also have an important influence

on the polymorphism. Other types of observation support this view. To get from somewhere south of the Pyrenees to the north of the continental range in Europe in 6000 years would require movement of at least 300 m yr^{-1} if the animals were marching in a straight line. After the recession of ice the molluscs, in common with plants and other animal groups such as beetles and grasshoppers, colonized newly available territory with remarkable speed (Atkinson *et al.* 1987; Hewitt 1996). We do not know how the movement was achieved, but the evidence that it can occur may indicate that passive displacement is much more common in snails than the careful ecological studies would suggest.

A number of surveys have now been done on *Cepaea*, using Wright's *F*-statistics or variances of allele frequencies at enzyme loci, to characterize the degree of genetic differentiation between populations. Ochman *et al.* (1983) and Selander & Ochman (1983) argue that, although some allozymes may be subject to selection, most alleles are more or less neutral, and that the patterns observed indicate periodic reductions to small numbers, accumulation of genetic differences through drift and subsequent expansion of territory. There is no reason to believe that the comparatively high correlation between samples is owing to selection over all the enzymes examined.

The theory of the behaviour of groups of populations linked by migration derives from Wright, and the parts considered here are discussed, for example, by Crow (1986), Hartl & Clark (1989), and Gale (1990). The approximate relations involved are as follows. A procedure adopted by Wright may be used to make enzyme data, for which there are several alleles at each locus, comparable with pairs of visible morphs (Cook 1992). The frequency of one allele is compared with the rest and mutation thought of in terms of forward and reverse mutation to and from the chosen allele. For a two-allele system with an allele at frequency q , inbreeding is related to variance as $F = V/(q(1-q))$. In practice, q is the mean for the samples in a defined area or region in the generation examined. Starting from $F_0=0$, the coefficient in generation n is $F_n = 1 - (1 - 1/(2N_e))n$. If populations are completely isolated and a balance of mutation and drift has been attained, resulting in a stable frequency distribution, then $8N_e u = (1-F)/F$, where u is the mutation rate and mutation is assumed to be similar in the forward and reverse directions. This equilibrium is approached very slowly. Wright's multi-allele island model assumes that there are many populations, and that migration from any one to any other distributes different mutations between them. On that basis, $4N_e m = (1-F)/F$ at equilibrium. $N_e m$ is the absolute number of migrants, and this relation demonstrates that it is migration, rather than mutation, which determines the structure of the system when m is much greater than u . If there is less than about one migrant per generation the populations behave as if they are distinct and tend to become homozygous; if there are more they tend to be heterozygous and the populations behave as a single system. The inbreeding coefficient is the usual F_{ST} and is the difference in heterozygosity between individual populations and the whole ensemble, divided by the heterozygosity of the whole (Nei 1987; Crow & Aoki 1984).

Table 3. Number of migrants, Nm , moving between colonies per generation, estimated from surveys of enzyme polymorphisms in *Cepaea nemoralis*

(Calculations based on geometric means of estimates from mean and variance of data for each locus (V) or from mean values of F supplied (F); those derived from variance have been adjusted for sample size (Weir & Cockerham 1984).)

region	Nm	data source
S. England, within areas (V)	1.9	Johnson (1976)
England & Wales (V)	0.2	Johnson (1979)
North Wales (F)	1.1	Jones <i>et al.</i> (1980)
Pyrenees (F)	2.1	Jones <i>et al.</i> (1980)
within region, Pyrenees (F)	2.4	Caugant <i>et al.</i> (1982)
within valleys, Pyrenees (F)	1.2	Ochman <i>et al.</i> (1983)
within regions, Britain and Europe (F)	1.1	Ochman <i>et al.</i> (1987)
French Pyrenees, populations within zones (F)	2.6	Valdez <i>et al.</i> (1988)
within regions, N. Spain (V)	1.9	Vicario <i>et al.</i> (1989)
within region, Pyrenees (V)	0.3	Guiller & Madec (1991)
France and Iberia, within regions (F)	1.2	Guiller & Madec (1993)

With complete panmixia between k populations each of size N , the time to get halfway to equilibrium is approximately $\ln 2 / (4u + 1/(2Nk))$ generations (Crow & Aoki 1984; and see Takahata & Nei 1984; Crow 1986). However, when there is a finite number of populations each linked by migration the inbreeding coefficient moves rapidly towards equilibrium. The equilibrium is $4N_c m = (1-F)(k-1)^2 / Fk^2$. This expression is not affected by changing the number of alleles assumed to be present, or by mutation rate (Crow & Aoki 1984; Crow 1986). The time to get halfway to it is $\ln 2 / ((2m+1)/(2N_c))$ generations, or $0.28N_c$ generations if $N_c m = 1$.

Some of these expressions are approximations, obtained by assuming that some terms of small expected value may be neglected. They also assume that migrants are equally likely to come from any population in the group, under which conditions migration is up to twice as effective as movement between adjacent populations (Crow & Aoki 1984). The surveys of enzyme variation were made in regions measuring hundreds of square kilometres, in which sampled populations are up to several kilometres apart. Table 3 shows estimates of Nm , the absolute number of migrants from one population to another, derived from the survey results. The estimates are fairly consistent, and indicate that one or two individuals move between populations per generation, or twice that amount if most movement is between adjacent populations. If the populations are assumed to be completely isolated and long established, the quantity labelled m would in fact be estimating the mutation rate, and effective population sizes would be one, two or three orders of magnitude larger than colony size indicated by ecological studies.

The data are mostly for regions such as river systems in the Pyrenees. One of the studies (Johnson 1979) is from samples collected widely over England and Wales, another (Guiller & Madec 1991) is for samples in the

Pyrenees which are rather distant from each other. These produce low estimates. In an ideal world with island migration or a long elapsed time, the distance between samples should not effect the result. However, if there are in fact selective patches for some loci or the founders are different in different regions or local neighbourhood migration has given rise to patterning, then lower estimates are to be expected when the collecting sites are spaced out. Differentiation at the enzyme level does not involve occurrence of unique principal alleles (Ochman *et al.* 1983), which supports the idea of long-distance migration.

In Ochman *et al.* (1983), F_{DR} (with a value of 0.1775) measures variation between demes within river valleys in a $180 \times 60 \text{ km}^2$ region of the central Pyrenees. If the variation reflects dispersion since the first founders arrived and effective population size is in fact small, then with $N=1000$ the rate of inbreeding is consistent with an elapse of 390 generations. On the same basis, the mean F -value for the Valle de Aran (Jones *et al.* 1980), a river system in an area roughly $35 \times 25 \text{ km}^2$, suggests about 220 generations since the first founders arrived. Ochman *et al.* (1987) note that their F -value for *C. nemoralis* within regions in Britain and Europe is close to that found in recently introduced *H. aspersa* in California (Selander & Whittam 1983). The latter species has a high level of passive dispersal by man. If a region of the Pyrenees can be colonized in a matter of centuries, then extensive movement occurs.

We may also interpret the result as indicating regular movement over the few kilometres between colonies. Approximately twice as many individuals must do so if migration is primarily between neighbours than if the island model applies. If colonies are small, then genetically speaking, they cannot be isolated. There is therefore either a high effective population number or rapid dispersal after frequent reductions in numbers or high average levels of movement after numbers are stabilized. Whichever explanation, or combination of them, most closely describes the true picture, movement plays a greater part in the evolution of morph frequency patterns than indicated by ecological studies alone.

(d) Time-scale

Cepaea species become adult in 1.5–2 years, and when adult, usually show about 50% annual survival, giving a generation time of between 2–3 years, depending on the contribution of animals in the older-age classes (Wolda 1970; Oosterhoff 1977; Cain & Cook 1989; Cain *et al.* 1990). Genetic change is likely to take many years to become appreciable, and even studies that are long-term from our point of view (Murray & Clarke 1978; Wall *et al.* 1980; Singh 1981; Cain *et al.* 1990; Cameron 1992; Arthur *et al.* 1993; Sacchi 1993) are at the lower limit for detecting and measuring significant changes.

C. nemoralis and *C. hortensis* live in a wide range of habitats, almost all of which have been subject to massive alteration as a result of changes in land use and agriculture over decades or centuries. In addition, climatic fluctuations over centuries or millennia have affected the species directly and through their effect on man. Rousseau (1987) examined a series of loess deposits in Alsace and compared them with another study from

central Europe (former Czechoslovakia). They are middle to upper Pleistocene in age, and the molluscan faunal composition reflects the changing climate over the period. He found that cold-temperate and dry-damp climatic axes could be established from what we know of present-day distribution and adaptation of the faunas. *Cepaea* was present (grouped with *Bradybaena fruticum* and *Arianta arbustorum*) in the drier, more temperate phases. During interglacials, the central European area received species from the south-east, while the Alsatian ones were characteristic of the present fauna and presumably came in from the south. Another study in Burgundy (Rousseau *et al.* 1992) concerned a series of deposits running from about 11 000 yr BP to the present. Faunal composition indicated that the oldest horizons were cold and dry, followed by a short warm, moist period about 9500 yr BP. The climate then became cooler and less moist to 5000–4000 yr BP. After that, the faunal composition suggested that the climate again changed. The authors propose that in fact removal of trees by man mimicked a climatic change, allowing open habitat and xerophilic species to colonize. *Cepaea* became part of the fauna at the end of this period, about 2000 BP.

In Britain, both *C. nemoralis* and *C. hortensis* first occurred in the Pliocene, disappearing when conditions became cold, to be followed by later reinvasions (R. C. Preece, personal communication; Gilbertson 1980). They were absent during the Late-Glacial, from 13 000–10 000 yr BP, but were certainly present by 9000 yr BP, while *Arianta* had established itself by about 11 500 yr BP (Kerney *et al.* 1980). They were abundant at the time of the climatic optimum (Currey & Cain 1968; Cain 1971) about 6000 yr BP.

For a given selection pressure change in gene frequency is slow at low and high frequencies. The standard equations show that for a dominant allele a shift in gene frequency from 0.2 to 0.8 takes approximately $5s^{-1}$ generations, where s is the selective coefficient, a shift from 0.05 to 0.95 taking approximately $21s^{-1}$ generations. With strong selection ($s=0.1$), and a generation time of 2.5 years, these figures translate to 125 and 525 years, respectively. The duration of a particular pattern of selection is therefore similar to the time required for a large change in gene frequency. Changes in habitat occur at random with respect to the snails so that there is a continuous lag in response. This may be one reason why associations of morph frequency and habitat are often suggestive but rarely allow firm conclusions to be drawn about selection.

4. A MODEL SYSTEM

Given the observations made here, can any more be said that would help to account for the maintenance of polymorphism? The effects of variable selection and migration between populations have now been thoroughly examined in terms of general theory, and can be shown in some circumstances to protect genetic variability (Maynard Smith 1970; Christiansen & Feldman 1975; Gillespie 1975, 1991; Felsenstein 1976; Hedrick *et al.* 1976; Spieth 1979; Karlin 1982; Nagylaki 1992). The problem is to relate theoretical results to particular biological examples and to appreciate what

kind of pattern will be generated. A simulation has been developed for this purpose.

Cepaea is assumed to have colonized northern Europe by moving northwards over the past 10 000 years, or about 4000 generations. The colonizers were probably initially polymorphic for banding, but with very high frequencies of yellow. Rare alleles at the ground colour locus were therefore dominant, and must sometimes have been advantageous in the new conditions, so that they could increase rapidly in frequency.

The model consists of a 50×50 grid of cells, or populations (or tesserae; Spieth 1979), each having 400 individuals. There is a pair of alleles, one dominant, the other recessive, at a locus, for which gene and morph frequency may be examined in each population in every generation. Forward and reverse mutation occurs with a probability of 10^{-5} per generation. If each population is separate from all others in such an array, increase in probability of fixation takes place at a rate determined by individual population size, countered by mutation. Runs of 4000 generations of the simulation return estimates of N , obtained from the variance of gene frequency in the 2500 populations and the known generation time, within 20 or less of the expected value of 400. The estimated variance is very close to the value expected in the absence of mutation. Mutation provides variety, should it be lost, but otherwise has a negligible effect over the time involved.

Exchange may take place between each population and the six surrounding it (stepping-stone migration controlled by a variable a_{mig}), whereas long-distance movement is simulated by a small random migration from any population to any other, with a defined probability of occurrence per generation (d_{mig} , similar to island model migration). To avoid edge effects, opposite borders of the grid are attached. When migration is included the fixation index initially increases at the rate determined by individual population size. It then fluctuates about a constant value for a long period, as migration tends to equalize the allele frequency between populations (Crow & Aoki 1984). Even small amounts of migration have a noticeable effect. Theoretical analyses have addressed various aspects of these relations (for examples, see Wright 1951; Crow & Aoki 1984; Takahata & Nei 1984; Slatkin & Voelm 1991; Nagylaki 1992; Chesser *et al.* 1993; Nath 1996; Whitlock & Barton 1997; and for a simulation study, Rohlf & Schnell (1971). These patterns were discussed for populations of *Cepaea* in Cain *et al.* 1990). Runs which start from $q=0.99875$ (one dominant allele per population), with substantial migration ($m>0.01$) but no selection, usually become fixed at $q=1$ as a result of drift, after about 100 generations. Starting at $q=0.5$, few or no populations become fixed in 4000 generations.

If these levels of movement occurred and an allele was established at some central mean frequency, then drift would not result in fixation in the time available since the Pleistocene. This result may be relevant to the banding locus, which was almost certainly polymorphic during the northward movement of *Cepaea*, but does not apply to the darker ground colours, which were absent or at low frequencies. Migration on its own has not been investigated more fully, because we are primarily concerned with the interaction with selection, for which a new set of

Table 4. Frequency distributions of recessive morph after 4000 generations in a 50 × 50 grid of simulated populations each of 400 individuals, with various migration values

(Selection changes at random every 20 generations in runs 1–12, and every 40 generations in run 12. Number of selective patches is 100 in runs 1–10, 25 in run 11 and 4 in run 12. The starting gene frequency is 0.5 for the first run, the rest start with 1 dominant allele per population. a_{mig} , migration rate to adjacent populations; d_{mig} , probability of 2% exchange with another population chosen at random.)

run	1	2	3	4	5	6	7	8	9	10	11	12
a_{mig}	0	0	0.015	0.03	0.06	0.015	0	0	0.06	0.03	0.03	0.1
d_{mig}	0	0	0	0	0	0.05	0.1	0.2	0.2	0.05	0.01	0.1
fixed	945	10	40	0	0	0	54	0	0	0	0	0
–0.1	0	0	89	486	462	410	1072	663	150	662	983	427
–0.2	0	0	14	355	480	263	217	395	388	449	402	171
–0.3	0	0	12	261	363	181	141	275	367	287	216	103
–0.4	0	0	9	228	257	160	105	191	317	195	156	205
–0.5	0	0	9	187	198	145	87	183	347	144	142	390
–0.6	0	0	12	154	161	133	69	153	252	132	128	436
–0.7	0	0	14	154	162	128	97	52	225	120	118	329
–0.8	0	0	19	143	139	185	98	153	213	114	90	282
–0.9	0	0	18	266	147	274	138	179	168	151	106	148
–1.0	170	373	349	265	121	534	326	153	73	217	152	9
fixed	1385	2117	1915	61	10	87	96	3	0	29	9	0

relations develops (for examples, see Gillespie 1975; Barton 1993; Zhivotovsky & Feldman 1993).

Selection favours or acts against the recessive morph, independently in each of a series of patches of contiguous populations. The number of patches may be varied from 1 to 100, or set at 2500, when each population is selected independently of all others. Selective values are picked from a random distribution, in such a way that the fitness of the recessive morph is $1-s$ if disadvantageous, or $1/(1-s)$ if advantageous. The distribution of s is roughly normal with a mean of zero. The variance in the runs allowed a 50% chance of s being as great as 0.05, so that while selection was often negligible it was sometimes substantial. After a number of generations fixed at 1, 20, 40 or some other figure, selection changes at random to represent the effect of habitat change. It is therefore always directional, but magnitude and direction vary randomly between patches and over time.

If there is no migration, and selection is different in each population but changes from one generation to the next, then the dispersive process simply increases. As the duration of a pattern of selection is lengthened the variance goes up, duration and variance being allometrically related. There is complete dominance and one morph is as likely to be advantageous as disadvantageous. The effect of selection is therefore asymmetric, as the dominant morph is more likely than the recessive to increase from a low frequency. When selection is present and strong, its effect clearly predominates.

The elements of the model can therefore be outlined as follows. A finite set of linked populations is set up, with a polymorphic locus which moves towards fixation. The eventual equilibrium brought about by mutation is irrelevant over the time-scale concerned. There are randomly fluctuating selection pressures, so that the effective number in each population is determined both by drift and selection variances. The same selection occurs in all the populations within a given sub-area (a patch), over which it has a homogenizing effect on

frequency. Migration leads to gene exchange between populations. The important observations to be made are: (i) the likelihood that a rare allele will move towards some central mean frequency in such an ensemble; (ii) the shape of the frequency distribution over time and the likelihood of a population becoming monomorphic; (iii) the approximate time-scale of polymorphism; and (iv) the coincidence of allele frequency and selective patch.

The simulations represent the ground colour locus, starting as almost monomorphic yellow (q near 1) but with a low frequency of the dominant pink allele. Morph, rather than allele, frequencies are given in all simulations, as that is all that can be scored in the field. Table 4 shows results after 4000 generations for a representative set of runs in which selection remains constant for 20 or 40 generations, then changes at random. The first run commenced at $q=0.5$ in each colony. The rest start with $q=0.99875$ (one dominant heterozygote per population), enough to ensure that the rare morph becomes established.

In runs 1 and 2 (table 4) there is no migration. Most populations become fixed, and the allele for which they are fixed depends on the initial frequency. The rest of the runs show results for a variety of combinations of a_{mig} and d_{mig} . When there is low migration (run 3, $a_{\text{mig}}=0.015$, $d_{\text{mig}}=0$; run 7, $a_{\text{mig}}=0$, $d_{\text{mig}}=0.1$) the effect of initial frequency is still apparent. For higher values, transient effects disappear and the mixture of selection and migration becomes effective in preventing fixation. As migration increases the distribution of frequencies moves from being U-shaped, with modes near to, but not at, fixation, to becoming a flat distribution of polymorphic populations.

It may be objected that migration rates are too high, or that the grid of populations represents a field situation too small to contain 100 selection areas. Polymorphism is still maintained when the number of selective patches is reduced (runs 11 and 12), and passive displacement may take place over long distances, so that the simulation could represent a pattern of widely spaced populations over an extensive area. Numerous networks of this type

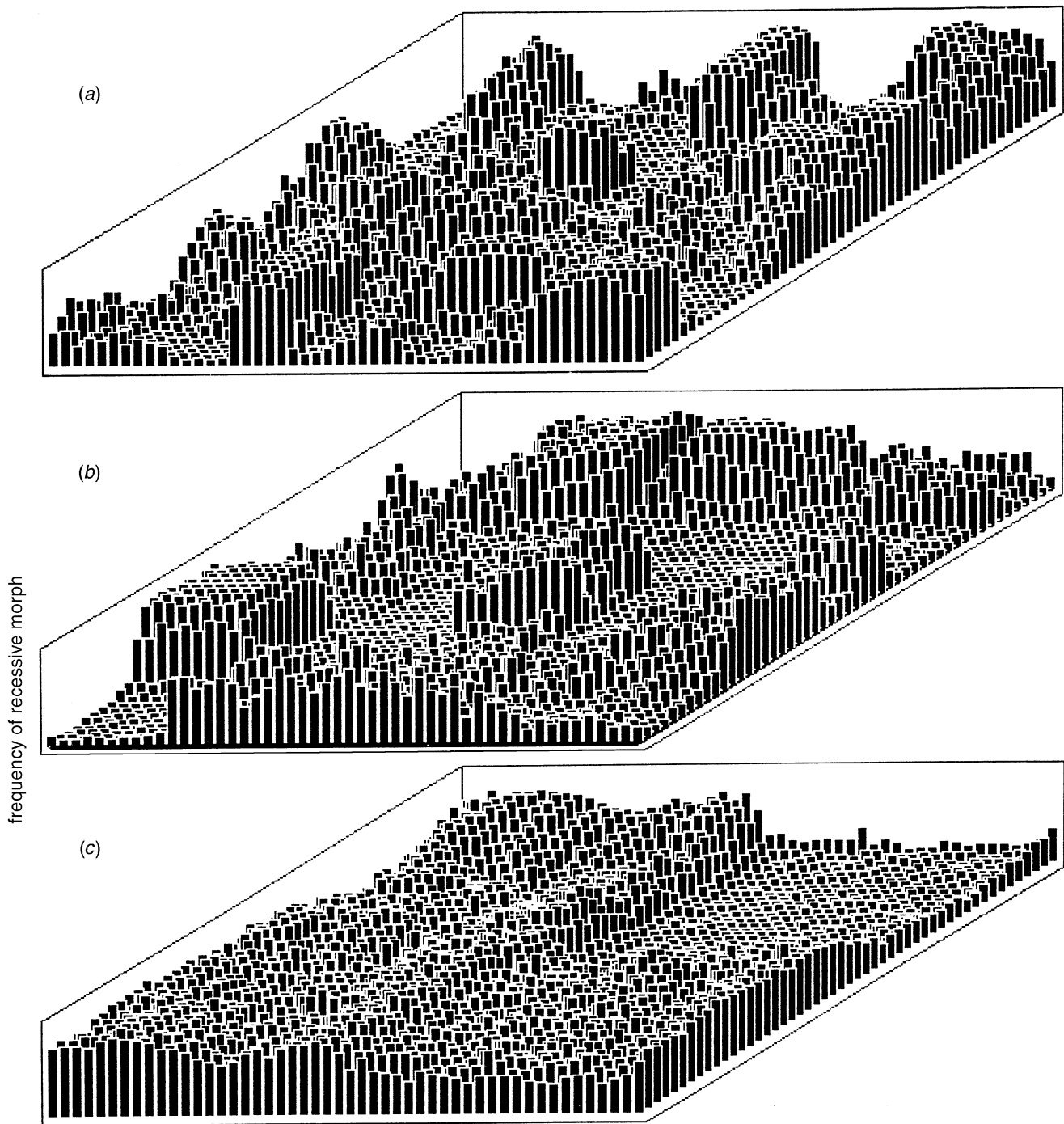


Figure 2. Distribution of morph frequencies in space after 4000 generations for runs (a) 10, (b) 11 and (c) 12 of table 4, having respectively 100, 25 and 4 selective patches. Frequency of recessive morph is shown on vertical coordinate.

would be linked by rarer migration from one to another to form the whole suite of populations of a region. Furthermore, the forces influencing frequency are interrelated; the important factor is the ratio of migration to selection, not the absolute values.

To make the model more realistic, individual populations should fluctuate in size and experience extinctions and invasions, patch size should vary with time, duration of selection in a patch should be a variable, variance of selection should perhaps be lower and migration also reduced. A metapopulation dynamics approach, as used by Pease and co-workers (1989) to examine environmental tolerance

and niche breadth, by Peck & Ellner (1997) to investigate the development of resistance when there is variable pesticide application, or by Akçakaya & Baur (1996) to model persistence and numerical fluctuation in *Arianta arbustorum*, might be adopted. Despite the shortcomings of the present model, however, there is a noteworthy result. A flat distribution of polymorphic populations readily arises, depending entirely on directional selection and movement, with no centripetal force built in.

Another relevant feature of the simulations may be seen when the distribution of morph frequencies in space is examined. Figure 2 shows three such patterns after

4000 generations. Although high and low frequencies clearly relate to the position and number of selective patches, there are sometimes both clines and areas of similar frequency that do not match the selective boundaries. The patterns sometimes look structured, as if following a topographical feature. The structure arises from migration and the history of changing selection in each patch, which cannot be unravelled from the final frequencies in the grid (cf. Peck & Ellner 1997).

5. CONCLUSIONS

Polymorphism may be present for two types of reason. Some force may operate which has a centripetal effect, moving gene frequency away from the extremes. It may be a frequency-dependent process which favours rare forms because they are rare; otherwise there must be a balance of some kind. In what is usually called the neutral situation balance is achieved by mutation from one state to another. The balance may also be between selection and mutation, between selective forces of different kinds, or between selection and segregation, which is how Haldane & Jayakar (1963) described heterozygote advantage. Relational balance, leading to heterosis, is a less secure way to arrive at the same end, strengthened by any process which reduces recombination. If there are clines such that high fitness in one habitat automatically entails low fitness in another, then balance of selection and migration also falls into this category, and is also possible if selection fluctuates at random. Most of the simulated runs tested had the arithmetic mean of fitness greater than 1 and the harmonic mean less than 1, which is a requirement for polymorphism in some spatial heterogeneity models (see, for example, Nagylaki 1992).

The second possible reason is that sometimes a polymorphism, once attained, persists for a very long time. It is a truism to say that a species only has a temporary existence, or that a given territory is only temporarily available to it. If the time-scale for loss of the polymorphism is similar to the time that the species has existed in a region, then there may be effectively permanent polymorphism although there is no true balance.

In *Cepaea nemoralis* and *C. hortensis* there are circumstantial reasons to believe that both heterosis and frequency-dependent predation help to maintain polymorphism for shell colour and pattern. There is evidence from a wide range of molluscs that visible polymorphism is associated with apparency and visual predation, and numerous studies have shown that predators feed in such a way as to protect polymorphisms in the prey. Many helicids are polymorphic. They are outbreeders which sometimes, at least, suffer inbreeding depression. These are good conditions for the visible characters to become linked into a balanced chromosome system. Both processes are centripetal in their effect and both could be very ancient, acting over periods much longer than the lifetime of a single species. Response to the two types of selection could act synergistically on the genome to produce adapted super-genes, but the time-scale involved must be very long and the selection may be weak. It is not easy to confirm or reject such a picture by population genetic studies.

The species with which we are concerned has existed in any given territory for no more than a few thousand generations. In that time it colonized new areas which were themselves in the process of change. It was probably polymorphic for banding at the start, but the present cline of increasing frequency of dark ground colours from south to north and the lack of ground colour polymorphism in two other extant species in the genus suggest the darker colours were initially rare and became established during the northward movement. Effective population size is larger than direct ecological measurements suggest, as a result of passive displacement. Selective pressures, including selective predation, vary widely from one habitat to another and are often strong. The habitats form a mosaic consisting broadly of woodland facies or open habitats. It may be that morphs adapted to one of these types are usually at a disadvantage in the other, in which case there is potentially a selection–migration balance. Whether or not that is so, the conditions the snails experience tend to retain the polymorphism over the time-scale concerned.

A two-stage model may therefore be proposed to account for the polymorphic patterns seen today. *Cepaea* species belong to a group in which evolutionary forces relating to breeding system and visual predation, operating over millions of years, have resulted in balanced heterotic genomes. Many types of habitat in which the species can live are likely to select visible differences in shell colour and pattern. Over the shorter timescale of thousands of years these give rise to polymorphisms which persist but are not necessarily truly balanced. When these persistent polymorphic states recur, they provide the genetic variability that has allowed a balanced system to develop. Populations studied in the field are probably almost never near equilibria and usually reflect only dimly the environmental conditions present at the place and time they are examined.

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