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Temporal morph frequency changes in sand-dune populations of *Cepaea nemoralis* (L.)

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Changes in morph frequency over time in sand-dune populations of *Cepaea nemoralis* in the British Isles have been examined using the Evolution Megalab database. Frequencies in colonies on a sand dune at Berrow, Somerset were estimated in 2008–9. This extends a survey started in 1926 by A. E. Boycott and C. Diver and continued in the mid-20th Century by B. C. Clarke and J. J. Murray. An increase in the frequency of the mid-banded morph, noted in earlier work, has continued. The apparent decrease in brown was not confirmed but the yellow frequency has increased. At a range of dunes in the British Isles, comparisons between data from the mid-20th Century and the early 21st Century indicate an increase in yellow and mid-banded morphs and a decrease in banding and brown morphs. These results differ from the overall trends derived from Europe-wide comparison of early data with Evolution Megalab data, in which banded and mid-banded increased in frequency, whereas there was no overall change in yellow. The general pattern of regional variation has been retained, although there is also high heterogeneity between samples, suggesting that a variety of factors are involved in explaining the changes. The mean shifts in frequency are consistent with climatic change over the period. Dunes are probably the most likely habitat in which to detect such a change. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, **108**, 315–322.

ADDITIONAL KEYWORDS: climate change – climatic selection – polymorphism – selective predation.

INTRODUCTION

The Megalab project (Silvertown *et al*., 2011) compared morph frequencies in *Cepaea nemoralis* (L.) in Europe using data from the mid-20th Century and a new series collected mostly in 2009. Overall, between the two periods, separated by approximately 15 generations, the frequency of the banded and mid-banded phenotypes increased, whereas there was a negligible change in yellow frequency. The data come from the full extent of the distribution of the species across the British Isles and Europe and therefore cover a wide range of frequencies, intensities of sampling, and climatic conditions. A generalized additive model was used in the analysis (Silvertown *et al*., 2011) to take account of these variables by including collection period, habitat type (wood, hedgerow, grassland or dune), altitude, precipitation, temperature, and variation in collecting between periods (referred to as location). Location, precipitation, temperature, and altitude all had significant effects. In an independent test of the influence of habitat, yellow frequency was lowest in woodland and higher progressively in hedgerow sites, grassland, and dune. Mid-banded and unbanded were less frequent in dunes than elsewhere.

The most detailed sampling in the two periods occurred in Great Britain, especially southern England, where altitude, precipitation, and temperature are comparatively invariant relative to the distribution as a whole. In exploring evidence for selection, it is therefore interesting to look further at the mean change between periods in the British subsection of the data. Are the changes seen there representative of the pattern as a whole as determined by the general analysis?

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On average, British populations of *C. nemoralis* on sand dunes have higher frequencies of yellow and many-banded shells and lower frequencies of mid-banded shells than are found in woods, hedgerows, and grasslands, confirming an earlier conclusion of Cain (1968). For Britain, coastal sand-dunes often experience relatively extreme climatic conditions. The general survey suggested that, unlike the other habitat classes, yellow may have increased on dunes. Within any dune system, there can be a variety of successional stages, from exposed mobile sand through various degrees of stabilization and plant cover to dense scrub and forest. Both natural events, such as extreme weather, and human modification of the environment (golf courses, building construction, erosion caused by visitors) may reverse succession, whereas sand accumulation may create new dune ridges and bury old ones. Frequently, dunes contain a shifting mosaic of different habitats, most of which are more exposed than habitats found inland. *Cepaea nemoralis* is often abundant alongside snail species such as *Cochlicella acuta* (Müller), *Helicella itala* (L.), and *Theba pisana* (Müller) that have xerothermic habitat preferences. *Cepaea nemoralis* cannot colonize the early mobile stage until stabilization by growth of grasses and broad-leaved plants has begun (Cameron *et al*., 1998). Subsequently, dense closed vegetation may eventually exclude it from consolidated dune, as may the lack of available calcium on sands composed mainly of silicates (Cameron, 2001).

Duneland populations of *C. nemoralis* were the subject of intensive and meticulous early studies by A. E. Boycott and Cyril Diver in the 1920s. Some of these populations were used to make detailed temporal comparisons by repeat surveys (Clarke & Murray, 1962a; Clarke, Diver & Murray, 1968; Murray & Clarke, 1978; Cameron, 2001). Surveys of other dune systems were also repeated (Arthur, Phillips & Mitchell, 1993; Cook & Pettitt, 1998). In some cases, net changes in morph frequency were recorded. Perhaps to a greater extent than in other habitats (Cook, Cowie & Jones, 1999; Cameron & Pokryszko, 2008), their interpretation can be complicated by changes in vegetation and by the possibility of local extinction and recolonization. In general, Boycott and Diver regarded morph frequency variation as evidence of random drift (Diver, 1932, 1939), whereas later surveys have tested for evidence of selection. In the present study, we used two sets of data to investigate temporal change in morph frequencies in dune populations of *C. nemoralis.* The first takes the results of multiple repeat surveys of populations on the dunes at Berrow (Somerset), the most detailed set available. The second uses new data entered in the Evolution Megalab database relating to dune systems reported by Cain (1968) around the British and Irish coasts to look for more general patterns.

MATERIAL AND METHODS

VARIATION ANALYZED

The characters considered for comparison are shell colour, the presence or absence of bands, and the band pattern modifier mid-banded. Brown, pink, and yellow are phenotypes controlled by alleles at the colour locus. The brown allele is the top dominant and yellow is the bottom recessive. In Britain, brown individuals are usually dark brown but, on dunes and in some other parts of the species range, there are pale brown individuals that may be difficult to distinguish from pinks; they are discussed further below. At a locus linked to the colour locus, unbanded is dominant to five-banded. Mid-banded removes four of the bands, leaving only the central one; it is dominant to non mid-banded and unlinked to colour and banding. There are other, sometimes less well-defined variants, although these are the only ones recorded in the Megalab database. Frequencies of mid-banded quoted in the present study are the fraction within bandeds.

The comparisons have been divided into two parts. The first involves the most complete study of temporal changes on dunes carried out at Berrow, Somerset (51.27°N, 3.01°W), first by Boycott and Diver and subsequently by Clarke and Murray, in which samples were located with a high degree of precision. As part of the Megalab survey, a set of samples was taken specifically to continue this sequence.

The second involves other dune samples taken by various collectors during the Megalab project that may be compared with data from Cain (1968). Because the exact location of the early collections is usually unknown, however, only general comparisons can be made between dates. Nevertheless, this might identify general trends.

Morph frequencies have been arcsin transformed for statistical testing. The data have then been tested for evidence of change in frequency and, where there is an apparent change, its magnitude has been estimated. The change in frequency over the sequence of collecting periods at Berrow has been examined using linear regression in analysis of variance (Sokal & Rohlf, 1969). For the Cain series, each comparison is between a pair of dates. The log cross-product ratio (odds ratio) has been used as a measure of the degree of change ($\log w = \log[m_2(1 - m_1)/m_1(1 - m_2)]$, where m_1 and m_2 are the early and late mean frequencies). The variance is $V_{(\log w)} = 1/(m_1.n_1) + 1/[(1 - m_1).n_1] + 1/(m_2.n_2) + 1/[(1 - m_2).n_2]$ for sample sizes n_1 and n_2. Then, for k samples, the weighted mean $w_{(m)}$ is the sum over k of $\log w/V_{(\log w)}$ divided by $V_{(m)}$, which is the

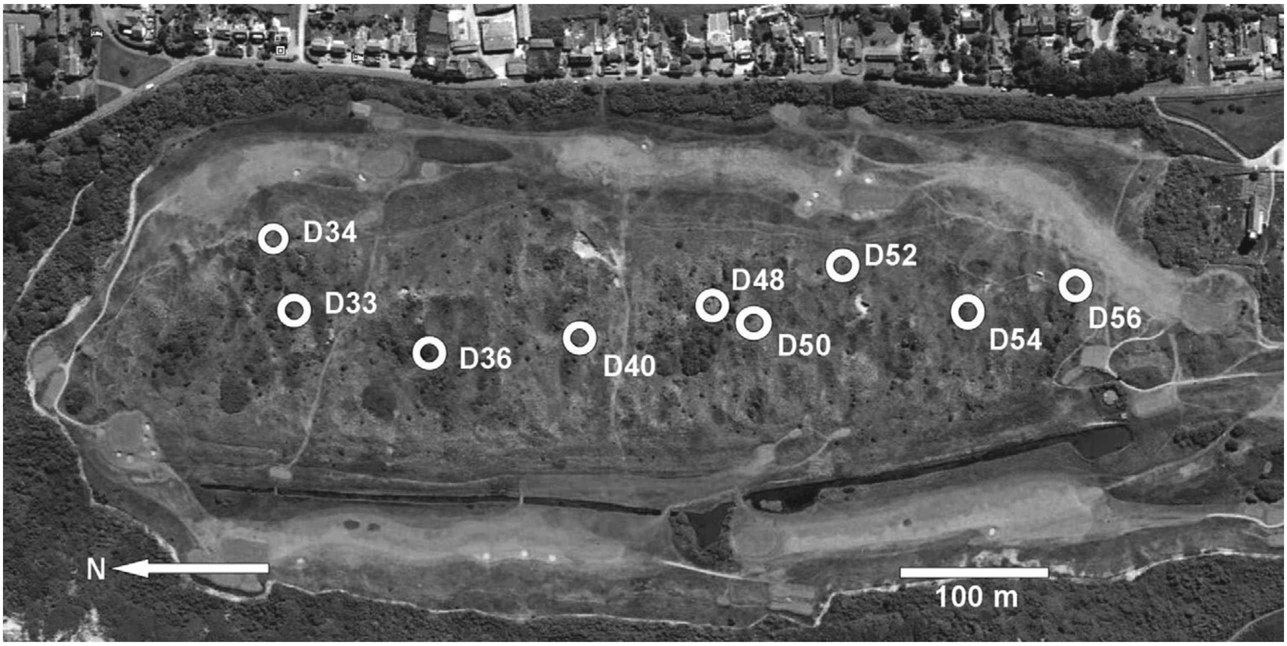

Figure 1. Distribution of nine samples sites on the sand dune at Berrow, Somerset; latitude 51.27°N, longitude 3.01°W. Image modified from Google Earth.

sum of $1/V_{(\log w)}$. The overall change in frequency can then be tested by treating the quantity $w_{(m)}^2/V_{(m)}$ as a chi-squared value with 1 degree of freedom. The sum of $[(\log w - w_{(m)})^2]/V_{(\log w)}$ is a chi-squared value with $k - 1$ degrees of freedom; if it is sufficiently large, the estimates display heterogeneity. An explanation for this is provided by Manly (1985).

Clarke & Murray (1962b) estimated selective coefficients at Berrow using a method that employs the definite integral over the number of generations elapsed of the difference equation that describes the change in frequency over one generation. Given the relative change in morph frequencies, the coefficient s estimates the amount by which one morph is fitter or less fit than the other; it may also be expressed as the fitness of one morph relative to unit fitness of the other, which is $1 - s$. Over a wide range of frequencies and differences in relative fitness, the cross-product ratio of morph frequencies has been found to be a good approximation to $1 - s$ over one generation and the n_{th} root of the cross-product ratio a good approximation when the frequencies used are separated by n generations (Cook *et al.*, 1999). This calculation has therefore been used with respect to the Cain series.

BERROW

Boycott and Diver surveyed the area in 1926, making 28 collections. Their results were published and the sites resurveyed on several occasions by Clarke & Murray (1962a, b; Murray & Clarke, 1978; B. C. Clarke and J. J. Murray, unpubl. data), who also extended the number of sampling sites. By 1975, they had noted pronounced habitat changes and an apparent increase in the mid-banded morph and a decrease in the brown morph.

In 2008, the nine sites for which there was the most complete data were recollected.

They had all been sampled in 1926 and on four subsequent occasions, in 1959/60, 1963, 1969, and 1975. They run along the length of the dune (Fig. 1) and, in 1926, had relatively low frequencies of brown and mid-banded in the north and higher frequencies in the south. There was little variation in pink frequency, so that yellow declined from north to south as brown increased. In 2008, the positions of the sites were assessed as accurately as possible using information supplied by Clarke and Murray and by comparing their published map with the image available from Google Earth. This allowed accurate relocation of sites because the original collecting areas were associated with identifiable features. Collections were made by Cook within areas approximately 20 m across for 30 min to 1 h.

Clarke and Murray noted that an unusual pale brown morph was present at Berrow, which is difficult to distinguish from pink; they therefore combined these two categories. When colours are pale, particularly with dead shells, there is some risk of mistakes in classification, especially when carried out by different people. However, mid-banded is clearly

Table 1. Frequencies of four phenotypes at nine sites in 2008 at Berrow, Somerset

Site	Total	Yellow	Brown	Banded	Mid-banded
D34	89	0.955	0.011	0.978	0.264
D33	108	0.991	0.000	0.981	0.085
D36	158	0.886	0.032	0.968	0.118
D40	93	0.860	0.043	0.957	0.180
D48	96	0.875	0.063	0.958	0.196
D50	90	0.867	0.100	0.911	0.305
D52	57	0.860	0.088	0.930	0.245
D54	123	0.911	0.081	0.935	0.209
D56	155	0.800	0.135	0.858	0.226

Mid-banded is expressed as a fraction of banded shells. Earlier data for the same locations are from Clarke & Murray (1962a) and Murray & Clarke (1978).

distinguishable from other banding types, and there is little chance of confusion with respect to dark brown.

The data for 2008 are listed on the Open University Evolution Megalab database (http://datadryad.org/handle/10255/dryad.35145). The frequencies of yellow, brown, many banded, and mid-banded are shown in Table 1. These have been compared with frequencies on the five previous occasions noted above, which have also been transcribed to the Evolution Megalab database. In 2008, few living individuals could be found in the more southerly sample sites (samples with higher code numbers). The surrounding area of golf fairways and greens is heavily managed. In the central rough ground where the sample sites are located there was sometimes evidence of damaged vegetation, possibly through use of herbicides. Where living snails were more abundant, they were often clustered in the leaves of *Iris foetidissima*.

COMPARISONS WITH CAIN'S SURVEY

The latitude and longitude given in the Megalab data for records from Cain (1968) serve to identify the dune concerned. As with *C. nemoralis*, in all habitats, frequencies often vary greatly over short distances. Comparisons with collections made in the later survey therefore cannot be made precisely. However, dunes are of finite extent. One way to examine the differences between dates is to compare all samples within a dune, without consideration of their exact location, where the recorded positions of early and recent samples differ by 10 km or less. This sometimes results in several samples for each period, so that there can be multiple pairwise comparisons. There is no way of choosing a best pair and no reason why a bias should arise from making an overall comparison. Consequently, the data presented are weighted means of the samples available from the early and late dates. Five late samples from Berrow (not included in the main analysis because they were not sampled in 1926) are compared with early records from the same locations. Samples on Cain's list from Scolt Head Island, Norfolk, are close to the location of some recent collections, although the earlier population has become extinct and the new samples are from a different section of dune not on the island (J. F. Peake, pers. comm.). These have therefore been excluded.

RESULTS

BERROW

The main objective of the 2008 sampling was to find out whether the trends previously observed by Clarke and Murray have been maintained. Progressive gene frequency dispersion would be expected under random drift. Under constant selection, the frequencies should follow curved trajectories that differ in shape depending on the initial frequency and the dominance of the morph. However, three different sets of collectors are involved: Boycott and Diver in 1926, Clarke and Murray for the main series, and Cook in 2008. They may have differed in searching technique and to a small extent in scoring colours. Re-collections may not be at precisely the same locations as previously and all samples are subject to random error. All these factors serve to obscure the finer details of frequency dynamics, so that, for statistical analysis of frequency change, it is reasonable to regard successive collections as independent samplings, which can be tested for evidence of trend using linear regression in analysis of variance. Table 2 shows results for tests on transformed frequencies of yellow, brown, banded, and mid-banded within banded. Using this procedure, mid-banded and yellow both show significant increase in frequency with time. Brown and banded do not change.

Because the data points in each regression are not strictly independent, it may be objected that this analysis will overestimate the significance of change. The most context-free approach would be simply to test the direction of change in frequency between each successive sampling within each colony. There are 45 such comparisons. For mid-banded, 32 are increases and 13 are decreases. The probability of this result or one more extreme is 0.003, so there can be no doubt that an increase has occurred in the mid-banded phenotype. For yellow, there are 24 increases and 21 decreases, with a probability of 0.38, which is not

Table 2. Mean morph frequencies for nine sites at Berrow at successive dates and the significance of a change in frequency

Character	1926	1959	1963	1969	1975	2008	$F_{5,48}$	P	$F_{1,48}$	P
Yellow	0.821	0.860	0.856	0.858	0.836	0.889	**0.58**	NS	**7.47**	0.003
Brown	0.085	0.054	0.036	0.052	0.044	0.061	**0.70**	NS	**0.17**	NS
Banded	0.930	0.936	0.976	0.961	0.971	0.968	**1.19**	NS	**3.31**	NS
Mid-banded	0.201	0.270	0.289	0.277	0.303	0.330	**2.20**	NS	**43.00**	< 0.001

The first F-value tests significance of variation in means between years; the second tests linear change in frequency with time. NS, not significant.

Table 3. Mean percentage frequencies of four morphs in mid-20th and 21st Centuries on dunes listed by Cain (1968)

Place	Cain samples	21st Century samples	Yellow	Brown	Banded	Mid-banded
East Scotland						
Gullane	1	1	41.3–61.0	31.0–2.4	60.1–46.3	0.7–3.5
North Berwick	3	1	26.3–71.3	30.9–3.0	58.0–85.6	0.0–5.2
East England						
Druridge Bay	2	1	55.5–89.2	0.0–0.0	95.1–86.2	0.0–0.0
Gibraltar Point	9	2	41.9–59.0	8.5–6.3	87.7–86.1	2.6–5.6
Central west England						
Ainsdale	3	7	30.0–31.4	0.0–3.7	100.0–98.3	19.7–33.0
West Wales						
Newborough	4	1	37.3–42.3	12.6–7.7	87.0–80.8	23.9–23.8
South-west England						
Berrow	5	5	59.8–79.1	3.0–3.3	98.5–95.5	8.4–25.7
Braunton	7	1	16.1–58.8	0.0–0.0	91.9–58.8	52.3–56.7
Crantock	1	4	99.3–89.9	0.0–0.0	94.0–100.0	36.4–20.9
North Ireland						
Portstewart	2	1	30.2–49.3	0.0–0.0	94.9–90.8	0.0–5.2
Magilligan Strand	1	1	22.5–87.1	0.0–0.0	97.5–100.0	0.0–26.9
Dunfanaghy	2	1	30.9–51.2	0.0–0.0	94.1–89.3	0.0–0.0
South-east Ireland						
Tramore Strand	4	5	79.1–65.3	0.0–0.0	86.2–89.5	0.0–0.2
Mean			43.9–64.2		88.1–85.2	
Trend χ^2 (d.f. = 1)			+95.31***	–27.88***	–1.12	+20.97***
Heterogeneity χ^2			344.73***	64.26***	108.52***	74.37***

Columns to the left give the number of samples available at each period. The other columns show the mean morph frequencies in the early and late series. The chi-squared testing trend shows the sign and significance of the direction of change. The chi-squared value in the last row measures the heterogeneity of the response between locations. ***$P < 0.001$. Data are from the Evolution Megalab database.

significant) and, similarly, the other comparisons are not significant when tested in this way.

Comparisons with Cain's survey

For each morph, the mean frequencies in Cain's surveys and the 2009 surveys are shown in Table 3. For each location, the chi-squared testing trend between the two periods has been calculated and is given a sign to indicate direction of change. The final row in Table 3 shows the chi-squared value, with 12 degrees of freedom, testing variation in direction of deviation between locations. Except for banding, the trend is significant, with yellow and mid-banded increasing and brown decreasing, whereas heterogeneity is significant in all cases. These values no doubt over-estimate the significance of the differences, although they also suggest that there have been real changes in mean frequencies, as well as heterogeneity between locations. The majority of changes in yellow and mid-banded are increases (12 in 13 and five in seven, respectively), and those in banding and brown are declines (eight in 12 and four in five). The slightly different treatment of the dune data by Silvertown *et al*. (2011) showed a significant increase in yellow, a just significant increase in mid-banded, and a nonsignificant decline in banding frequency. The frequencies exhibit some of the geographical patterning discussed by Cain, and the general correspondence between frequencies at the two dates shows that the dunes have retained their individuality. The lower entries in the table, where brown is absent, are from the south-west peninsula of England and from Ireland, areas that experience relatively high winter temperatures. Coolest winter temperatures occur in the group at the top of the table from Gullane to Gibaraltar Point.

STRENGTH OF SELECTION

Using the Berrow data, selective coefficients have been calculated for mid-banded and yellow. With six records of frequency at each site, there are 15 possible pairwise comparisons for which selection may be estimated, giving 135 estimates over nine sites. Any within-site pair of values is as valid as any other to estimate selection, so the weighted mean of all within-site selective coefficients has been calculated. Calculations were based on a generation time of 3 years (Clarke & Murray, 1962b). For the 135 comparisons available for mid-banded, the mean of s is –0.029 with an SE of 0.0025. The 130 comparisons for yellow frequency give $s = -0.019 \pm 0.0026$. Both estimates are significantly different from zero ($P < 0.001$), indicating advantages of approximately 3% and 2% per generation, respectively. The changes in banding and brown frequency are not significant.

For samples compared with those listed by Cain (1968), the significant increase in yellow corresponds to an advantage of 4% per generation over the period. Considering only those samples that are polymorphic at both periods, the increase in mid-banded corresponds to a 3% advantage, whereas the decline in brown indicates 6% selection against it.

DISCUSSION

THE NATURE AND CONSISTENCY OF CHANGES

Polymorphisms, and particularly those for which there is evidence for differences in fitness among variants in at least some circumstances, are useful for studying the balance of evolutionary forces at play in natural populations. Changes within populations over time are potentially a powerful tool in such studies, although there are practical and conceptual difficulties in their interpretation. Where there is insufficient direct evidence relating to any particular selective agent (almost always the case), selection is most convincingly demonstrated when several clearly independent populations change in the same way over time in parallel with some independently measurable trend.

In the present study, the changes at Berrow, monitored by several repeat surveys over 82 years indicate that both yellow and mid-banded frequencies have increased with some consistency, with selection coefficients in the range 2–4%. The change in mid-banded was detected in earlier surveys (Murray & Clarke, 1978).

Consideration of other detailed resurveys on dunes shows that these changes are not always repeated elsewhere. The nearest geographically is at South Haven Peninsula, Dorset, an area of acid soils, partly covered in shifting dunes (Cameron, 2001). Diver studied *C. nemoralis* populations there in the 1920s and 1930s. In 1999, Cameron resurveyed Diver's sites and mapped extensions of range. Most of the snails are pink banded, with a locally restricted minority of yellows and browns. There was no evidence of a systematic change in shell colour morphs. A substantial fraction of the colonies have mid-banded shells; 11 in 14 of them showed an increase in mid-banded frequency. Selection could be estimated in only 12, of which ten increased in frequency. The changes are heterogeneous; the nonsignificant coefficients measuring increase correspond to an advantage of approximately 2%, given a 3-year generation (estimates were calculated for a longer generation time in Cameron, 2001).

In 1924, Boycott and Diver collected samples of *Cepaea* from dunes at Bundoran, County-Donegal, in the west of Ireland. These dunes were resampled by Clarke and Murray in 1961 (Clarke *et al*., 1968), who found the morph frequency distributions similar to the earlier pattern with no evidence of evolutionary change. By contrast to the situation at Berrow, the dunes were much more constant in habitat (and probably with less seasonal variation in climate. At Berrow, the dunes became progressively more consolidated with thicker and more extensive vegetation.

The dune system at Point of Air, north Wales, first surveyed by Cain with P. M. Sheppard in 1962 (Cain, 1968), was re-sampled 35 years later by Cook & Pettitt (1998). Frequencies were very variable along its length, and the change in yellow relative to pink was in different directions in different parts of the system. Overall, yellow declined in frequency and mid-banded increased, whereas brown frequency showed no evidence of change. The estimated disadvantage to yellow was approximately 2%, and the advantage to mid-banded was 3–4%. Here, too, habitat changes took place, with removal of holiday accommodation, landscaping, and restitution of part of the dune after burial of a pipeline. At Seaton Sluice, Northumberland, Arthur *et al*. (1993) detected no change in morph frequencies over the shorter period of 16 years. There had been little alteration in habitat.

The picture is of heterogeneity among locations, with some significant changes in morph frequencies associated with changes in habitat, which are responses to local conditions, rather than to large-scale environmental change. When more general comparisons are made, however, some of the trends seen at Berrow appear to be widespread. Thus, the Megalab pairs analysis (Silvertown *et al*., 2011) and the data presented in Table 3 indicate an increase in yellow. The comparison of data in Cain (1968) from several widespread dunes with 21st Century samples from the same dunes suggests a 4% advantage to yellows over the second half of the 20th Century. Mid-banded similarly shows an overall increase, whereas banded and brown decrease. Despite an overall tendency for yellow to increase, declines are recorded from Crantock and Tramore Strand, and a decline in mid-banded at Crantock (Table 3). Sampling error aside, local conditions lead to individual differences.

MOVEMENT

Although concerted changes among independent populations cannot be a result of drift, the possible effects of movement need consideration. At the local level, Cook & Pettitt (1998) found evidence for homogenization of morph frequencies at Point of Air, and, away from dunes, Cook *et al*. (1999) detected a similar trend on the Marlborough Downs. In situations where one morph is the most frequent in most populations, migration will have the greatest effect on those few populations in which it is less common (the initial differences having arisen via past bottlenecks and isolation). It is probable that long distance movement has been underestimated (Cook, 1998) and, thus, that the independence of populations within the same system may be less than assumed. If adjacent colonies have levels of exchange of more than one or two individuals per generation, there is a marked increase in effective population size with a consequent increase in correlation of sub-units. Hence, it is important that some of the changes reported in the present study are found across many systems far apart from each other.

CAUSES OF CHANGE

If we accept that these consistent changes are products of selection, we are left to consider the agents involved. Climate and visual selection by predators have both been used to explain particular patterns of variation, although direct evidence of their effects is sparse. Over the period covered by the present study, there has been a large change in mean climate, the effect of which might be particularly apparent on dunes. Cain (1968) argued that the high frequency of banding typical of dunes and, in many cases, the high frequency of yellow, was consistent with visual predation. However, he also showed that high brown frequencies were associated with structural complexity and suggested that browns may be more resistant to cold conditions generated by ponding of cold air in complex habitat features. In addition, mid-banded was characteristic of southwest trending slopes at Point of Air and various exposed English downlands, whereas both yellow and mid-banded are frequent in France in open country (Lamotte, 1959; Cain, 1968) and in south-east Poland, which experiences a relatively extreme climate (Ożgo, 2005). The general increase in yellow from north to south over the range of the species (Jones, Leith & Rawlings, 1977; Silvertown *et al*., 2011) similarly suggests that yellow is associated with higher temperatures. Local climatic effects may therefore operate to influence frequency. A warmer climate, with more insolation, might also favour shells lacking the upper two bands, including mid-banded (Lamotte, 1959). The effects of predator selection are harder to assess. Cain (1968) attributed the generally high frequencies of yellow and five-banded shells on dunes to their being the most cryptic in this exposed but rather variegated habitat. At Berrow, Murray & Clarke (1978) showed that a reduction in rabbit populations was associated with a reduction in short turf, which has been replaced by longer, coarser grasses and herbs. At the same time, sea buckthorn *Hippophae rhamnoides* has increased greatly in extent, reducing the area suitable for *Cepaea* but providing protection for its main predator, the song thrush *Turdus philomelos*. When taken together, the increase in cover (decreasing exposure of the snails) and variegation should favour five-banded shells on both hypotheses. We note, however, that there has been a general decline in song thrush populations over the period involved (Cameron & Pokryszko, 2008). All the changes recorded in the present study (i.e. an increase in yellow, a decrease in bandeds as a result of an increase in both unbanded and mid-banded, and a decrease in brown) could result from climatic change, which has led to higher summer temperatures (affecting yellow and banding) and milder winters (affecting brown). These effects would be superimposed on local factors tending to generate heterogeneity among dunes.

Overall, the results emphasize the problems that arise when looking for change in a broadly dispersed data set. A multitude of possible interacting causative agents could be involved, arising from changes in agriculture, forestry, urbanization, pollution, and predator densities. It is not unexpected that patterns are difficult to extract. More intensive studies of particular habitats or locations are necessary to increase precision, such as those carried out at Berrow, on the Marlborough Downs (Cowie & Jones, 1998), at Rickmansworth (Cameron & Pokryszko, 2008), at Deepdale (Cameron, Cook & Greenwood, in press), and in the Netherlands (Ożgo & Schilthuizen, 2011). If the results are indeed a result of climate change, then the increase in yellow and mid-banded is predictable from increasing environmental temperature.

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