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Frequency of *insularia* during the decline in melanics in the peppered moth *Biston betularia* in Britain

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Over the last three decades the frequency of the dark melanic form *carbonaria* of the peppered moth *Biston betularia* has declined in Britain. Data have been examined which show the intermediate phenotype *insularia*, controlled by alleles at the same locus, to have increased or remained level in frequency. Phenotype frequency of *insularia* does not always track allele frequency accurately because it is recessive to its alternative when *carbonaria* is common but dominant to the alternative when typical is common. It is shown that if *insularia* fitness lies between that of *carbonaria* and typical, and melanics replace typicals or vice versa, there will be a rise and fall in *insularia* allele frequency during a transitory period. The path followed is strongly affected by initial gene frequencies. During the high melanic period in Britain, differences in *insularia* frequency between localities may have been influenced by history of arrival of the novel morphs as well as by local selective conditions.

Keywords: *Biston betularia*, industrial melanism, *insularia*, multiple alleles, peppered moth, selection.

Introduction

New information on morph frequency in the peppered moth *Biston betularia* has recently become available, and some earlier conclusions about this example of evolution have been challenged. The nature of the criticisms is discussed by Grant (1999) and Cook (2000). One feature which has always received comparatively little attention is the status of the intermediate melanic morphs. The darkest melanic, known as *carbonaria*, is a uniform black colour except for two small white marks at the base of the fore wings. Forms which are intermediate in colour between this and the typical phenotype are known as *insularia*. At least five alleles are involved altogether, each dominant to paler alleles in the series. These phenotypes are illustrated by Kettlewell (1973), Lees & Creed (1977) and Bishop & Cook (1980). The different *insularia* alleles segregate in progeny of crosses, but often cannot be separated into discrete categories in collections of wild insects (Steward, 1977). It is therefore not always clear whether more than one phenotype is present in a sample, or which phenotype is intended in the scoring used by different observers. In the past, *insularia* has been up to about 40% in South Wales and Gloucestershire, where it was sometimes at a higher frequency than *carbonaria*, but never rose above 15% elsewhere (Kettlewell, 1973).

Where *carbonaria* was at high frequency, *insularia* was usually rare, so that when describing the recent dramatic drop in frequency of *carbonaria* in industrial areas it has been appropriate to leave the intermediate phenotypes out of the discussion (e.g. Clarke *et al*., 1994; Grant *et al*., 1996; Cook *et al*., 1999). In this paper some more recent data on *insularia* are presented, and the patterns of change in *insularia* frequency are discussed.

Results

Data

When Kettlewell (1973) reviewed the data on *insularia* he had available a small amount of evidence from the time of increase in melanics in the 19th and early 20th centuries and from 1950 to 1970, when frequencies across the country were more or less unchanging. He concluded that three patterns were evident, which had different geographical distributions. In the Severn Valley region of western England and South Wales, *insularia* was at 15–40% and *carbonaria* at a lower frequency. In the south of England *insularia* and *carbonaria* were both at low frequencies, while in the highly industrialized parts of England *carbonaria* was at a high and *insularia*

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Table 1 Data relating to *insularia* frequency.

(a) Changes in morph frequency (%) in *Biston betularia* at some English sites. The final column gives the estimated *insularia* allele frequency, calculated as $\sqrt{(B + C)} - \sqrt{C}$, where B and C are the phenotype frequencies of *insularia* and *typica*. Data for Manchester from Bishop *et al.* (1978) and Cook *et al.* (1999), Nottingham from K. Cooper, York from T. J. Crawford, Kent from West (1994), Hampshire from Kettlewell (1973) and Siggs (1978 and earlier)

	Date	*carbonaria*	*insularia*	*typica*	Total	Allele
Manchester area	1952–64	98.5	1.5	0.0	760	0.123
	1968–74	95.9	1.7	2.4	2421	0.048
	1990–98	20.8	5.2	74.0	289	0.030
Nottingham	1993	58.8	5.9	35.3	272	0.048
	1994	50.4	7.0	42.5	228	0.052
	1995	38.5	8.2	53.3	353	0.054
	1996	27.3	6.7	66.0	373	0.040
	1997	28.9	4.1	67.0	318	0.025
	1998	30.9	4.3	64.8	256	0.026
	1999	19.2	5.1	75.8	99	0.029
	2000	19.0	6.0	75.0	100	0.034
York	1990	57.9	10.5	31.6	19	0.087
	1991	61.8	2.9	35.3	34	0.024
	1992	74.1	0.0	25.9	27	0
	1993	69.6	4.3	26.1	23	0.041
	1994	60.0	0.0	40.0	15	0
	1995	75.0	0.0	25.0	8	0
	1996	51.4	8.1	40.5	37	0.061
	1997	42.1	10.5	47.4	19	0.073
	1998	41.2	11.8	47.1	17	0.081
	1999	37.0	0.0	63.0	27	0
	2000	20.8	12.5	66.6	24	0.073
Kent	1971	78.2	7.6	14.3	119	0.090
	1975	76.6	13.1	10.3	107	0.163
	1979	71.7	11.1	17.2	99	0.117
	1983	64.7	16.7	18.6	102	0.163
	1987	54.3	20.7	25.0	164	0.176
	1990	42.3	23.1	34.6	104	0.171
	1991	30.9	41.2	27.9	165	0.303
	1992	33.5	29.2	37.3	236	0.205
	1993	23.0	31.1	45.9	74	0.200
	1994	23.8	33.8	42.5	80	0.221
Hampshire	1957–64	7.1	2.3	90.5	518	0.012
	1965–70	5.8	5.8	87.3	689	0.031
	1973–77	6.5	6.5	87.0	352	0.034

(b) Regression of *insularia* phenotype frequency on date, *carbonaria* frequency and *insularia* allele frequency for four locations. Values of *t* measure difference from $b = 0$. Those for Kent and Caldy are significant

		Date		*carbonaria*		*allele*	
	Generations	*b*	*t*	*b*	*t*	*b*	*t*
Kent	10	1.206	5.62	−0.465	−7.37	1.771	8.33
Nottingham	6	−0.356	−1.22	0.035	0.77	1.081	4.22
Caldy, Wirral	39	0.089	5.94	−0.030	−4.72	0.843	6.71
York	10	0.183	0.32	−0.182	−1.66	1.214	3.06

at a low frequency. In the 1950–70 period *insularia* sometimes appeared to change in frequency. At Kettlewell's home site in Oxfordshire the frequency of typical dropped roughly from 50% to 40% whereas *insularia* increased from 15% to 25% and *carbonaria* remained more or less constant. At Newbury, Berkshire, *insularia* also increased at the expense of typical while at Minstead, Hampshire, a small increase in *insularia* coincided with a corresponding decline in *carbonaria*.

As *carbonaria* dropped in frequency in the latter part of the 20th century, *insularia* often increased. At Caldy, on the Wirral peninsula, the frequency of *carbonaria* changed from over 90% in the 1960s to less than 10% by the end of the century (Clarke *et al.*, 1994; Grant *et al.*, 1996). At the same time the frequency of *insularia* rose from about 1% to about 3.5% (Mani & Majerus, 1993; Grant *et al.*, 1996). Although this change is small it is significant (Table 1b). An equally spectacular change in *carbonaria* frequency occurred in Manchester. It has not been as well recorded and different sites have been used in different surveys, spread over the area of the conurbation. Nevertheless, pooling results listed separately in Bishop *et al.* (1978) and Cook *et al.* (1999) provides figures which appear to show a similar increase in the *insularia* morph (Table 1a). Mani & Majerus (1993) give data for sites in Cambridge, Cambridgeshire, Northwood, Middlesex, and Egham, Surrey, where the *insularia* frequency rose from 5% or less in the mid 1960s to about 15% by 1990, while *carbonaria* declined from about 80% to about 35%. Data for Nottingham and York are shown in Table 1a. For Nottingham *insularia* appears unchanged at about 6% from 1993 to 1999, while *carbonaria* declined from 59% to 19%. At York the *insularia* frequency was between 5% and 10% in 1990–2000, while *carbonaria* has declined from 60% to 30%. At a site in Kent *insularia* rose in frequency from about 7.5% in 1971 to more than 30% by 1994, while *carbonaria* dropped from over 75% to between 20% and 30% (West, 1994). Where change is evident the general pattern is for *insularia* to be constant or to increase in frequency, while the darker melanic morph *carbonaria* has experienced a decline.

If fitness of *insularia* were intermediate between that of the other two morphs it should increase in frequency when *carbonaria* was common, because it is relatively advantageous, but decline as the most favoured morph, *typica*, becomes the most common. During the original period of increase in melanics *insularia* presumably rose in frequency and then declined in localities where *carbonaria* was the most favoured form. Mani (1980) has shown that these patterns may be simulated in a theoretical model based on ecological data, and that patterns in specific locations can be effectively reproduced (Mani & Majerus, 1993). His model runs for a period of 150 generations, and includes changing selection imposed by sight-hunting predators, estimates of selection arising from developmental or metabolic differences, and migration. Under the circumstances it is not transparently clear which components contribute to the relative change in frequency of the intermediate melanic phenotypes. It is therefore of interest to consider the expected deterministic change when the additional ecological considerations are ignored. The dynamics of multiallelic systems have been examined in the past (Owen, 1954; Kimura, 1956; Mandel, 1959; Li, 1967), but to investigate stable equilibria rather than the consequences of directional selection.

Analysis

Two considerations arise when we examine probable patterns of change in *insularia* frequency. The first results from the dominance relations of the alleles. If *insularia* is present in a population largely composed of *carbonaria* it is recessive to the alternative allele and only homozygotes are distinguishable. In one composed mostly of typicals it is dominant and heterozygotes are distinguishable. As *typica* replaces *carbonaria* the visibility of *insularia* alleles changes. If the gene frequencies of *carbonaria*, *insularia* and *typica* are p, q and r ($p + q + r = 1$) the system is as follows,

Phenotype		A			B	C
Genotype	cc	ci	ct	ii	it	tt
Frequencies	p^2	2pq	2pr	q^2	2qr	r^2.

The effect of the change in dominance of the alternative alleles is most easily seen if we assume a population containing the three morphs, in which, for the sake of the demonstration, the *insularia* allele remains unchanged at frequency q. Frequency of *typica*, r, may vary from zero to $1 - q$, the remainder being *carbonaria* alleles at frequency $p = 1 - q - r$. The *insularia* phenotype frequency is $B = q^2 + 2qr$. The slope of B on r is 2q, and $B = q$ when $r = \frac{1}{2}(1 - q)$. Suppose earlier events have reduced r to a low frequency, and it is now increasing under selection. When it is low the *insularia* phenotype frequency will be less than the allele frequency, but it will become greater than q when $r > \frac{1}{2}(1 - q)$. Systematic change in prevalence of the *insularia* phenotype will occur, for which we might be tempted to suggest a selective basis, whereas the effect is due to nothing but the genetic background against which the allele is apparent.

The second consideration is that under selection *insularia* allele frequency is affected by the change in relative frequency of the other two alleles. The simplest demonstration of the basic dynamics of the situation can be made by imagining a haploid system with three genetically controlled colour types. This hypothetical model is introduced to avoid the effect of segregation of alleles into genotypes, although, of course, it has no biological relevance to Lepidoptera. As before, *carbonaria*, *insularia* and *typica* are assumed to have allele frequencies p, q and r, so that

genotypes and phenotypes are	*c*	*i*	*t*
with frequencies	p	q	r
and fitnesses	$1 - s$	$1 + k$	$1 + s$.

Fitnesses are assumed to be related to appearance, that of *i* lying between *c* and *t*. When typicals have highest fitness s is positive. The mean fitness is $\bar{w} = 1 - s(p - r) + kq$. Change in frequency of the *insularia* allele over one generation is,

$$\bar{w}\Delta q = sq(p - r) + kq(p + r)$$

It is evident that when the darker melanics are very common, so that p is larger than r, Δq is positive and q increases. When typicals are very common, so that r exceeds p and q is small, Δq is negative and q decreases. When $\Delta q = 0$,

$$q = 1 + 2sp/(k - s)\ .$$

This equation describes a straight line from $q = 1$, $p = 0$ to $q = 0$, $p = \frac{1}{2}(s - k)/s$. At $q = 0$ the frequency p is zero when $k = s$ (*insularia* has the same fitness as *typica*), and 1 when $k = -s$ (*insularia* has the same fitness as *carbonaria*). When k lies between these limits the trajectory for *insularia* has a maximum. When $k = 0$, so that *insularia* is exactly intermediate in fitness between the other morphs, $p = 0.5$ at $q = 0$. The maximum is then independent of s. Where a given trajectory crosses this line depends on frequencies at the beginning of a period of selection. The equation for Δq shows that if two populations had the same initial frequency q, and were subject to the same selection, the frequency in the next generation is determined by their respective values of $p - r$. This quantity can have a wide range of values subject only to the restriction that $p + r = 1 - q$, and will in turn determine the change in subsequent generations.

In the simplest possible case of directional selection, therefore, a genotype with intermediate phenotype and fitness shows an initial rise and subsequent fall in frequency in whichever direction the change occurs. The rate of change depends on strength of selection and frequency, but the path followed and the peak frequency are strongly dependent on initial frequencies of the three genotypes.

To illustrate the true diploid case we will assume that fitness differences are related to visual appearance and $k = 0$. We then have

Genotypes	cc	ci	ct	ii	it	tt
Frequencies	p^2	2pq	2pr	q^2	2qr	r^2
Fitnesses	$1 - s$	$1 - s$	$1 - s$	1	1	$1 + s$.

The mean fitness is $\bar{w} = 1 - s[p(2 - p) - r^2]$. The changes in *carbonaria* and *insularia* frequencies are

$$\bar{w}\Delta p = -sp[(1 - p)^2 + r^2]$$

$$\bar{w}\Delta q = sq[p(1 - p) - r^2]$$

The expression for Δq changes in sign as r moves from being very rare to very common, while that for Δp does not. The *carbonaria* and *insularia* morphs change in frequency in opposite directions at one extreme and in the same direction at the other, whether melanics are favoured or disfavoured. The maximum at which $\Delta q = 0$ occurs at

$$q = 1 - p - \surd p(1 - p)$$

This is a curve running from $p = 0$, $q = 1$, to $p = 0.5$, $q = 0$. If k is introduced to modify the fitness of *insularia*, and allowed to vary between s and −s, the curve changes in position in a similar way to that for the haploid model. Figure 1 shows possible frequencies of q plotted on p. On the diagonal border $r = 0$, whereas r is greater than 0 in the body of the diagram, moving towards 1 at $p = 0$, $q = 0$. The curve running from $p = 0$, $q = 1$, to $p = 0.5$, $q = 0$, defines $\Delta q = 0$. Representative trajectories are illustrated for four populations starting from the same frequency q but with different frequencies p and r.

If there were two *insularia* alleles with fitnesses related directly to appearance in the way described, then both would show the rise and fall in frequency, with the less extreme form changing in frequency after the more extreme one. For the haploid case, suppose the four types have frequencies p, q, r and u, with fitnesses $1 - 2s$, $1 - s$, 1 and $1 + s$. Changes in the two *insularia* types are described by

$$\bar{w}\Delta q = sq(p - q - 2u)$$

$$\text{and } \bar{w}\Delta r = sr(2p + q - u).$$

For the darker morph, change is positive or negative depending on the frequency of *carbonaria*, on the one

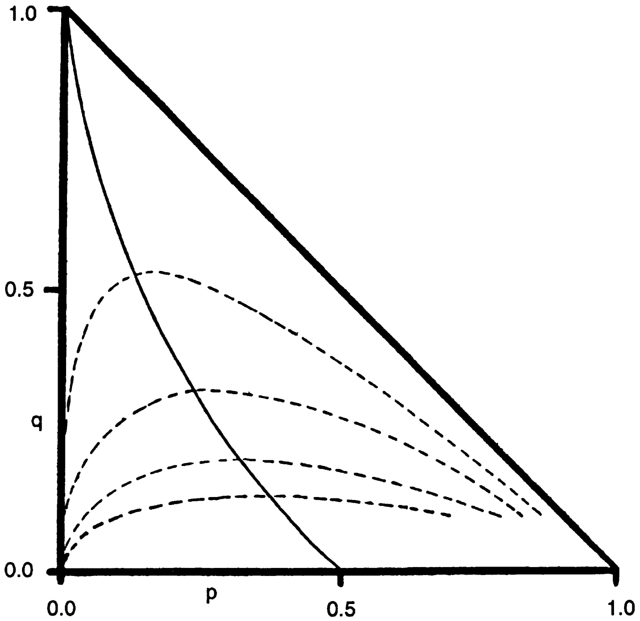

Fig. 1 Relation of frequency of the *insularia* allele (q) to that of the *carbonaria* allele (p) in populations which also include the typical allele (frequency of typical = 1 − p − q). Fitnesses of *carbonaria*, *insularia* and *typica* are 1 − s, 1 and 1 + s. Solid line: $\Delta q = 0$. Pecked lines: trajectories for populations moving towards $p = 0$, $q = 0$, starting from the same value of q but different values of p and r. With this model the paths of the trajectories do not depend on s.

hand, and of pale *insularia* and *typica* on the other. For the paler morph the change depends on the ratio of *carbonaria* and dark *insularia* to *typica*.

Discussion

Of the 12 examples of changing frequency considered here, the *insularia* phenotype increased in frequency in nine and was more or less stable in three. The recorded change from Manchester (Table 1) illustrates the changing detectability of the *insularia* alleles. The allele frequency declines from 0.12 to 0.03, while the phenotype frequency increases from 1.5%, when most of the rest were *carbonaria* to 5.2% when most others are *typica*. In all other cases discussed the relation of allele to phenotype frequency is positive, significantly so when more than one or two records are available. Phenotype frequency therefore correctly indicates the direction of change, although not necessarily the precise amount.

Like Manchester, the Nottingham site is moving in the expected final direction of loss of all nontypical alleles; the range of values is beyond the theoretical Δq maximum and both *insularia* and *carbonaria* are declining (Table 1). When examining change in *carbonaria* frequency Grant *et al.* (1998) compared this site with one at Chesterfield, Derbyshire. Experience from field surveys shows that patches of similar frequencies are large, except where there are marked clines, and this site was acceptably close. In Kettlewell's (1973) list no *insularia* were recorded there in 1952–56 (99.5% *carbonaria*, total = 170) and 0.9% in 1967–8 (96.6% *carbonaria*, total = 293, allele frequency of *insularia*, 0.026). The *insularia* frequency therefore appears to have risen to its peak between then and the commencement of the sequence in Table 1.

In two cases discussed by Kettlewell which start in the 1950s the *carbonaria* frequency was stable over the period, while an increase in *insularia* was compensated for by a decline in typical. The data from Hampshire in Table 1 look more or less unchanging. Mani & Majerus (1993) illustrate data from this county in which the *carbonaria* frequencies are consistent with these; they do not show the *insularia*. In the rest, *carbonaria* declined, sometimes to a very great extent. In none of these examples has *insularia* been separated into subcategories, but it is possible that sometimes more than one *insularia* allele is involved. Brakefield & Liebert (2000) provide evidence from the Netherlands of different *insularia* phenotypes showing different changes during a decline of *carbonaria* frequency.

Examination of the dynamics of a system of three or more alleles with directional selection shows that the observed changes in *insularia* are usually consistent with a fitness lying between that of *carbonaria* and *typica*. This, in turn, is consistent with the assumption that fitness is directly dependent on visual appearance, although of course that assumption is not essential. The actual path followed at any given site is affected by numerous factors, such as changes in selection over time, migration from sites with different frequencies and regional differences in characteristic vegetation or kind of resting sites available. It is therefore not appropriate to attempt further analysis of the fitness relationships.

An additional and interesting complication is that the frequency achieved after a period of time may be strongly influenced by initial frequency. The order in which the different melanic phenotypes first entered an area could have a marked effect of frequencies 50–100 generations later, even in localities where similar selection operated. A difference in frequency at the time of the main Kettlewell surveys may therefore reflect initial conditions as well as, or instead of ecological differences. Equally, differences in response over the last three decades may reflect differences in frequencies at the beginning of the present decline in melanism, as well as local selective conditions. Consideration of the fate of *insularia* adds another dimension to the study of this example of extremely rapid genetic change in response to changing natural conditions.

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