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The *medionigra* gene in the moth *Panaxia dominula*: the case for selection

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SUMMARY

Analyses of changes in frequency of the gene *medionigra* in colonies of the moth *Panaxia dominula*, begun by R. A. Fisher, E. B. Ford and P. M. Sheppard, have long been regarded as a model study of natural selection under field conditions. Recently, their conclusions have been criticized, on the grounds that phenotypes have been improperly scored and that population structure has been misunderstood. The results are re-examined here, including recent unpublished collections.

It is argued that the colonies studied are distinct populations, as usually defined, and that the results could not arise as a result of migration. Fluctuation in population size from year to year, large variance in fecundity and some features of mating behaviour probably reduce the effective number to less than half the estimated population size. Variable expressivity and consequent subjective variation in scoring, casts some doubt on earlier claims that selection fluctuates significantly from generation to generation, and on a reported case of increase in frequency from a very low starting point. However, the one natural and three artificial colonies studied are consistent in providing estimates of selection of 7 per cent or more against *medionigra*, despite the variation introduced by small population size and scoring difficulties. There appears to be an equilibrium at a low frequency, and the rate of approach to it suggests that the selection is frequency dependent. One artificial colony, at West Kirby on the Wirral, Merseyside, U.K., does not show an equivalent decline. Selection cannot be as strong as indicated elsewhere, but since the population size is small some disadvantage to *medionigra* cannot be rejected.

1. INTRODUCTION

In Britain the scarlet tiger moth *Panaxia dominula* is a predominantly day-flying arctiid moth, which lives in more or less isolated colonies in the south of England and Wales. It has an annual life cycle, the adults flying between the end of June and early August. The fore wing is black with iridescent green structural colouring and a pattern of cream or white spots. The hind wing is usually bright scarlet with black markings. It has long been of interest to collectors and as a result variant forms in which the wings have more or less black on them than usual, or the red on the hind wings is replaced by yellow, have been noted from time to time in one or other of the colonies (Cockayne 1928; Kettlewell 1942; Robinson 1971).

An allele producing a phenotype called *medionigra* when heterozygous and *bimacula* when homozygous was discovered at a site near Oxford known as Dry Sandford or Cothill, and named by Cockayne (1928). When he did so, the generic name of the species was *Callimorpha* Latreille, 1809. It was changed to *Panaxia* by Tams (1939) on a question of priority, a name then used by Kloet & Hinks (1945). Franclemont (1950) produced an argument for the retention of *Callimorpha*. This case is not recorded in Melville & Smith (1987), but Kloet & Hinks (1972) reverted to the earlier usage, and a number of recent papers have been published under that name. Most of the genetical studies have used the name *Panaxia*, however, so that has been retained here. The moth played a significant part in the history of population genetics as a result of a study begun by Fisher & Ford (1947). They used a method of mark, release and recapture, which they had developed to estimate at the same time the gene frequency, the survival rate and the total adult population size each year in the Cothill colony over a number of generations. Armed with this information, they determined the expected fluctuations in gene frequency, and showed that the actual fluctuations from year to year were much greater than expected if the population estimate was a reasonable indicator of effective population size. The population varied from about 1000 to 7000 over the period studied, and because it was small and the apparent selection strong, Fisher and Ford (1947) were led to claim that their findings were 'fatal to the theory which ascribes particular evolutionary importance to such fluctuations in gene-ratio as may occur by chance in very small isolated populations'. Of course, that is an overstatement of the case. Wright (1948) was stimulated to produce a rebuttal, his point of substance respecting the Cothill population being that effective population size may be much smaller than the number of individuals estimated to be flying, so that the fluctuations in gene frequency may indeed have been largely due to sampling error (see Wright 1978; Provine 1985). Other studies were begun on the

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initiative of Sheppard (1951, 1953 and cited below), and the moth became a model for the study of gene frequency change in natural populations.

Sheppard (1953) countered Wright's argument about effective population size by showing that the ecology of the insects was not likely to lead to accidental loss occurring in the way Wright suggested, the eggs of this species being non-adhesive and spread widely by the females. At the same time he showed with a longer series of data (Sheppard 1951) that the changes in gene frequency which Fisher and Ford had been studying constituted a systematic decline, so that continuous selection against the *medionigra* gene in heterozygotes or homozygotes or both was strongly indicated. This led him to establish a number of colonies into which the gene was introduced, in order to follow changes under conditions other than those at Cothill (Sheppard 1956; Sheppard & Cook 1962). One of these, at a site at West Kirby on the Wirral peninsula, Merseyside, was started in 1961 with larvae of backcrosses of *medionigra* to typical. This site is now known as 'Wirral Way', since what was once a railway line has become a footpath of that name. No results from it had been published by Sheppard at the time of his death in 1976, and its continued existence was unsuspected until Clarke, who was sampling night-flying moths nearby, obtained a stray *bimacula* individual from the colony in 1988 (Clarke *et al*. 1990). Since then, gene frequencies in the colony have been estimated in each subsequent generation by Clarke *et al*. (1993, 1996). Although gene frequencies at Cothill dropped, consistent with selection against the gene, the results for West Kirby show levels similar to those originally introduced, suggesting a different pattern of selection there. The frequencies recorded by Sheppard earlier in the history of the colony have now come to light (Clarke *et al*. 1996), and later records are available for some of the other artificial colonies. They are used in this paper to review the evidence on frequency changes in the *medionigra* gene in different colonies.

Since the work of Ford and Sheppard a number of criticisms have been made of the original results. Clarke *et al*. (1993) have expressed doubt that the moth is colonial, while Owen & Clarke (1993) concluded that, because of mis-scoring and the effect of temperature on expression of phenotype, the primary data may be faulty. Owen & Goulson (1994) claimed that the interpretation of data on *medionigra* frequency as evidence for selection is invalid. It is therefore impossible to consider the frequency of the *medionigra* gene in colonies of *Panaxia dominula* without giving attention to the definition of a colony and the expressivity of the gene.

2. BACKGROUND

(*a*) *Boundaries of colonies*

There is no doubt that *P. dominula* is locally distributed. In the survey published by Kettlewell (1942) it was described as living in colonies, and in order to assist other lepidopterists some early collectors described particular localities where it was to be found. Localization is sometimes effected by the restricted nature of suitable habitat, for example, the small area of alkaline fenland lying between agricultural fields at Cothill. Where suitable territory is more widespread, for example, along canal banks in parts of southern England, it tends to be centred on favoured food plants, especially species of comfrey (*Symphytum* spp., Boraginaceae: further information on food plants is given by Cook 1961 and Owen 1994).

White (1985) argued that where suitable territory is extensive, the behaviour of the species, including courtship in which females fly little and assemble males to them, will lead to local concentrations of high density, these centres sometimes moving from one place to another. Numbers in a given place may fluctuate greatly from year to year, probably as a result of fluctuations in weather conditions or local ecology, so that what are usually separate sites may join and exchange individuals. Although colonies can shift in position, those recorded by Kettlewell (1942) and revisited by Cook (1961, 1962*a*), over 20 years later, showed high site specificity. In the U.K., the moth is confined to southern England and south-to-mid Wales (Cook 1959); Cook (1961) argued that both the limited distribution and the tendency to specialize on *Symphytum* are related to the sensitivity of overwintering larvae to the effects of the British climate. The Cothill colony is near the present northern edge of the range for natural colonies.

Ford (1971 and earlier publications) described the Cothill colony as isolated. As Owen & Clarke (1993) and Owen (1995) have pointed out, however, it is possible for migrant insects to move between it and other colonies, especially the nearest about 2 km distant at Sheepstead Hurst. In a recent year of extremely high density, moths spread out from the centres and have been found between the two colonies (Owen 1995). Nevertheless, records since Cothill was first studied in detail in 1941, as well as the surveys of the region by Sheppard over many years and his failure to find moths at apparently suitable sites such as the one at North Hinksey where he established an artificial colony (Sheppard 1951, 1953, 1956), show that such conditions are uncommon and sporadic. As Wright (1978) says, colonies are isolated to an extent that seems complete in the short run, though their very existence shows that diffusion occurs in the long run. Although migration could affect the evolution of the species by introducing a gene to a population where it did not previously occur, there is no evidence that this has happened at the sites surveyed. The changes in gene frequency observed are large, and could only be brought about by movement of individuals from one colony to another if migration was so common that the sites would no longer appear to be separate colonies (Cook 1993; Jones 1993*a*). For present purposes, therefore, colonies may be considered to be real and bounded, usually with negligible migration from one to another.

(*b*) *Expression of the* medionigra gene

The forms *bimacula* and *medionigra* were first described by Cockayne (1928) as coming from Cothill. Breeding

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showed that they are the homozygous and heterozygous expressions of an allele at a single locus, referred to here as the *medionigra* locus. Many other breeding studies have confirmed this conclusion, especially those by Sheppard, who reared large numbers of animals to provide stock for releases in artificial colonies. The *bimacula* form is so-called because all but the two basal cream spots of the fore wing are absent or reduced, while on the hind wing the separate black markings of the typical form coalesce into a stripe. The location of the missing fore-wing spots is often apparent, however, because structural iridescence is not affected by the mutant and is lacking wherever there are usually pale markings. The heterozygote *medionigra* has a less extreme expression, with a central cream spot on the fore wing reduced or absent and a black spot appearing in the hind wing. Expressivity is variable in both genotypes, however (Kettlewell 1942). In the heterozygote the central fore-wing spot may be hardly affected or the hind-wing black spot barely present, these features varying independently. When expression is very slight the hind-wing spot can be reduced to an area of yellowish scales on the red background, no black pigmented scales being present.

So far as we know, Cothill is the only natural locality in Britain where *medionigra* has ever been at all common, although phenotypes similar to it with a hind-wing black spot are found in the region where *P. d. dominula* and the Italian subspecies *persona* meet in northern Italy, and are characteristic of the subspecies *pompalis* from the eastern end of this border (Kettlewell 1942; Cook 1962*b*). Ford (1971) argued that the lack of dominance and variable expressivity are characteristics to be expected of a newly arisen mutant, and he states that he modified expression by selection in different lines towards complete dominance and complete recessiveness (Ford 1960; Ford & Sheppard 1969). By crossing *medionigra* to typicals from other colonies Kettlewell and Sheppard obtained individuals that had a more extreme expression than those usually found at Cothill, and some of these were used to found artificial colonies. Illustrations have been published showing the range of expressions that can be exhibited (Fisher & Ford 1947; Ford 1971; Jones 1989, 1993*b*; Clarke *et al.* 1991; Owen & Clarke 1993).

Darkening of the wings by reduction of fore-wing spots and presence of black in sensitive parts of the hind wings may also be induced in *P. dominula* by raising the pupae at higher than normal temperatures. Kettlewell (1944) obtained extreme expressions in *medionigra* by keeping the pupae at a mean of 21 °C, and typicals can also be made darker in this way. Owen & Clarke (1993) report frequencies from reared samples that are much higher than those they obtained from the field from two colonies, presumably as a consequence of the rearing conditions; Owen & Goulson (1994) offer further data showing expression of *medionigra* to be more extreme at higher temperatures. This is in accord with the reaction norm effect frequently seen in lepidopteran wing-pattern variants, as described by Goldschmidt (1955; Goldschmidt had earlier worked with melanic variants of *dominula*). The effects of temperature during pupation on lepidopteran wing patterns are reviewed by Nijhout (1991). Kettlewell (1944) also found a gene segregating in a colony at Deal, Kent, which produced an expression similar to that of the *medionigra* heterozygote, but only when the pupae were raised at high temperature. He also (Kettlewell 1946) states that a specimen seen, but not collected, by him from a colony in Devon was definitely *medionigra*. Occasional specimens from other colonies have yellow scales or a black spot, often asymmetrical, on the hind wings.

The phenotypes of the *medionigra* gene are therefore brought about by darkening of particular areas of the wings, which may also be affected by the environment during pupation. The sensitive parts of the wings are probably also influenced by other mutant genes, and are the typical pattern in individuals from other subspecies. The problem in the present context is that nearly recessive *medionigra* are almost identical to typical, and some typicals may have an environmentally induced phenotype like *medionigra*. The true status of such doubtful individuals could only be determined by progeny testing, which is impossible for any long-term field survey.

A single investigator is likely to achieve scoring consistency, so that a change in frequency from generation to generation will be correctly recorded. If several different investigators are involved at different times, however, there may be fluctuations in recorded frequency due to change of convention. This problem has been noted by Lees (1970), Clarke *et al.* (1991), Owen & Clarke (1993) and Jones (1993*b*), all of whom have sampled populations previously studies by Ford and Sheppard. However, the evidence for the systematic change in frequency at Cothill over the first few generations was monitored by Ford and Sheppard; when other collectors were involved in later years the frequency apparently fluctuated between limits of a few per cent, so that any influence of scoring bias there has been low. In this paper we examine the data for consistent changes, and since these are almost always downwards from a score by Ford or Sheppard to those recorded by another investigator, adoption of the most inclusive definition of *medionigra* for the later period will minimize the chance that an apparent trend is due to a change of scoring.

3. MATERIALS AND METHODS

(*a*) *Gene frequency of* medionigra *in various colonies*

(*i*) *Wirral Way*

When Sheppard died in 1976 his research notebooks were passed for safe keeping to the American Philosophical Society archives in Philadelphia. It is these that have provided the early records (Clarke *et al.* 1996). The relevant Sheppard papers consist of day books with notes of the different genotypes and sexes as they were caught, and estimates of population sizes. The colony was started by releasing 13 000 larvae from backcrosses of typical to *medionigra* in 1961. In the following year six *bimacula* were caught in a sample of 316. These are accepted by Sheppard but noted with a

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query; they should not be there and indicate either that some contaminants from other crosses were accidentally released with the backcrosses, or that some heterozygotes were so extreme as to be indistinguishable from the *bimacula* homozygotes.

Samples taken by Sheppard were used in most years for a mark, release and recapture analysis. Estimates of total seasonal emergence are therefore available. These figures, made using the Fisher and Ford method and spanning 1962 to 1967, are shown in table 1, with the samples examined by the Clarkes from 1989 to 1995. They are shown graphically in figure 1. The figure given for 1976, when no population estimate was made, is in the same ratio to sample size as the average estimate-to-sample size for the earlier samples, giving a population size of 101. The later series consist of some individuals observed in the field and some bred from larvae taken just before pupation (details are given in Clarke *et al.* 1990, 1993, 1996). Evidence of the ratio given above and the numbers observed on the wing suggests populations of between 100 and 200 for this period. Wherever possible, population estimates are listed for other colonies, to give some idea of abundance and expected fluctuations.

(*ii*) *Cothill*

The gene frequency before 1928 was guessed to be about 1.2 per cent (Kettlewell 1942; Fisher & Ford 1947). In 1939, when the detailed work started it was 9.2 and in 1940 11.1 per cent. Estimates of gene frequency and population size were published by Fisher and Ford (1947), Sheppard (1951, 1953, 1956) and Ford & Sheppard (1969), and have been gathered together and extended by Jones (1989, 1993*b* and this paper). Owen and Clarke (1993) give further estimates for 1991 and 1992. These figures are summarized in table 2, and the pattern from 1939 onwards is illustrated in figure 2.

Table 1. *Records of the medionigra gene in the Wirral Way colony*

Data from Clarke *et al.* (1990, 1993, 1996). Population size estimates are by Sheppard, using the Fisher & Ford method and represent the total emergence for the season. The value in parentheses is obtained using the mean ratio of sample size to estimate for the five earlier estimates. Collections to 1976 by P. M. Sheppard, later ones by C. A. Clarke and F. M. M. Clarke.

date	typical	*medionigra*	*bimacula*	gene freq	population size
1961	13000 backcross larvae			0.25	
1962	149	161	6	0.274	
1963	136	76	15	0.234	603
1964	92	72	13	0.277	275
1965	31	14	0	0.156	144
1966	27	25	0	0.240	116
1967	14	17	2	0.318	97
—					
1976	31	5	1	0.095	(101)
—					
1989	36	27	5	0.272	
1990	112	35	5	0.148	
1991	76	52	1	0.209	
1992	71	30	2	0.165	
1993	59	47	0	0.222	
1994	50	36	0	0.209	
1995	38	34	2	0.257	

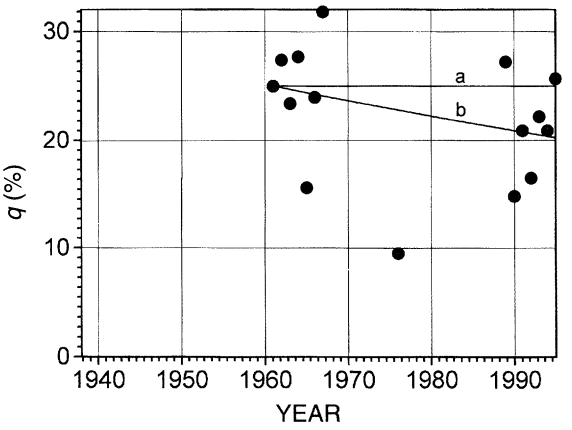

Figure 1. Frequency (per cent) of the *medionigra* gene in the Wirral Way colony, an artificial colony at West Kirby, Merseyside, established in 1961 at a frequency of 25 per cent *medionigra*. The points represent estimates from samples summarized in table 1. The two lines indicate the average change from the starting value expected if the selective disadvantage of *medionigra* were respectively (a) zero or (b) 0.8 per cent (selective coefficients of 0.0 or 0.008). The lower line is the maximum likelihood estimate from the data.

(*iii*) *Ness and the Genetic Garden*

The so-called Genetic Garden attached to the former Zoology Department building in Oxford was used to provide food plants for a variety of experimental insects and for outdoor insect and snail rearing. It contained comfrey (*Symphytum* spp.), which brought with it larvae from Kettlewell's own stocks, which included the *medionigra* gene. Details of the frequency of the three genotypes for four generations, 1958–1961, are given by Sheppard & Cook (1962). The frequencies are shown in table 3 and figure 3. Stock from the Genetic Garden, which had *medionigra* strongly expressed, was used by Sheppard in founding later colonies. Sheppard & Cook (1962) provide information for a single generation in an artificial colony started at Ness, on the Wirral peninsula. Eggs from matings of *bimacula* with typical were put down in 1959, and in 1960 the emerging adults were augmented by eggs from crosses between *medionigra* individuals. The following year 36 typical, 42 *medionigra* and 21 *bimacula* were captured; the expectation in the absence of selection was a 1:2:1 ratio. Sheppard's field notes include a reference to one *bimacula* caught, one seen and three *medionigra* caught in 1963. In 1964 one typical insect was seen and a further release was made of larvae from a cross of typical with *bimacula*. No other information is available and the colony appears to have become extinct shortly afterwards.

(*iv*) *Hinksey*

A colony at North Hinksey, on the outskirts of Oxford, was established by Sheppard in 1951, when he

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Table 2. *Typical and* medionigra *alleles in samples from Cothill and mean estimated seasonal population size*

Data from Ford (1971), Jones (1989), Jones (1993) and unpublished. Many collectors were involved. The names of those known are noted below the table. Owen & Clarke (1993) provide further data for 1991 and 1992.

date	typical alleles	*medionigra* alleles	gene freq	population size
1939	405	41	0.092	—
1940	208	26	0.111	—
1941	859	63	0.068	2250
1942	388	22	0.054	1600
1943	508	30	0.056	1000
1944	947	45	0.045	5500
1945	696	48	0.065	4000
1946	1888	84	0.043	7000
1947	2582	100	0.037	6000
1948	1863	69	0.036	3200
1949	987	29	0.029	1700
1950	2300	88	0.037	4100
1951	1133	29	0.025	2250
1952	2934	108	0.036	6000
1953	2122	56	0.026	8000
1954	2261	67	0.029	11000
1955	623	7	0.011	2000
1956	2538	78	0.030	11000
1957	3076	148	0.046	16000
1958	2664	102	0.037	15000
1959	939	21	0.022	7000
1960	371	7	0.019	2500
1961	337	7	0.020	1400
1962	45	1	0.022	216
1963	117	1	0.008	470
1964	62	0	—	272
1965	160	2	0.012	625
1966	74	0	—	315
1967	100	0	—	406
1968	259	3	0.011	978
1969	1054	38	0.035	5712
1970	919	31	0.034	449
1971	1283	9	0.007	7084
1972	675	5	0.007	3471
1973	461	1	0.002	1500
1974	1683	11	0.007	2500
1975	112	2	0.019	< 1000
1976	337	3	0.009	1500
1977	24	0	—	< 500
1978	79	1	0.013	< 1000
—				
1988	1238	6	0.005	4500
1989	1615	19	0.011	6500
1990	1043	7	0.007	9000
1991	4098	34	0.008	45000
1992	4535	45	0.010	60000
1993	943	23	0.024	6000
1994	444	8	0.018	1500
1995	276	8	0.028	1250

Known contributors to collecting:
1938–46, E. B. Ford with R. F. Bretherton, R. A. Fisher, W. H. Dowdeswell, T. C. Carter, C. I. Rutherford; 1947–1956, P. M. Sheppard; 1954, Q. Bone, M. H. Williamson; 1955, Q. B.; 1956, E. B. F., M. H. W.; 1957, no details; 1958, E. B. F., J. V. Z. Brower, L. P. Brower, L. M. Cook, E. R. Creed, K. G. McWhirter; 1959, L. P. B., L. M. C., E. R. C., E. B. F., K. G. M., P. M. S., H. E. Paterson; 1960, C. J. Cadbury, L. M. C., E. R. C., E. B. F., D. A. Jones, H. C. Jones; 1961, no details; 1962, L. M. C., E. B. F., R. J. A. Metcalfe, ?. Chalmers; 1963–65, E. B. F., J. M. & E. Mayr, L. B. Rea, S. Whalley; 1966, E. B. F., D. R. Lees, V. Scali; 1967, E. B. F., D. R. L., L. E. Gilbert, Th. Dobzhansky; 1968, E. B. F; 1969, D. R. L., P. T. Handford, A. Shapiro; 1970, E. B. F., D. R. L. alternate days & P. Brunet; 1971, D. R. L., C. Gibson; 1972, E. B. F., P. B., D. L. T. Conn, C. G., ?. Chandler; 1973, E. B. F., D. R. L; 1974–78, E. B. F. (no other names in records); 1988–95, D. A. J., H. C. J.

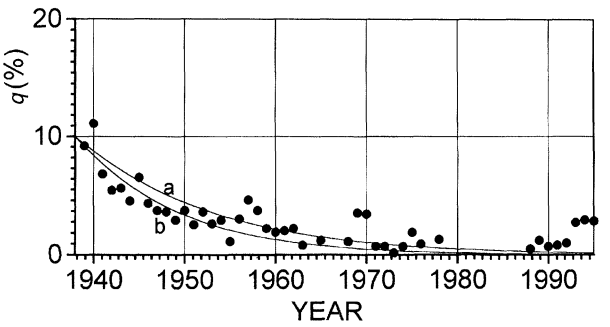

Figure 2. Frequency of *medionigra* in the natural colony at Cothill, near Oxford. The curves are for the periods (a) 1939–91 (7.1 per cent disadvantage; $s = 0.071$) and (b) 1939–56 (9.3 per cent disadvantage; $s = 0.093$). Data given in table 2.

was studying the Cothill colony. He released 4000 eggs of the backcross typical × *medionigra*, so that the expected gene frequency in 1952 was 25 per cent. Only 30 adults were recorded, which had a *medionigra* frequency of 15 per cent. Numbers remained low over the next few generations, and the next available sample is from 1959. Results to 1961 are given by Sheppard & Cook (1962). Sheppard's field books provide estimates for 1964, 1965, 1966 and a larval sample for 1970. A result for 1991 was obtained by Jones (1993*b*) and for 1991 and 1992 by Owen & Clarke (1993). These records are assembled in table 4 and shown graphically in figure 4.

(*v*) *Sheepstead Hurst*

This is a natural colony about 2 km from Cothill. Sheppard sampled there from 1949 (Sheppard 1951) and made estimates of the population size for a number of years. By 1954 he had records of 11 102 insects, none of them *medionigra*, and in that year he released the eggs from 50 backcrosses of *medionigra* to typical (Sheppard 1956). The estimated population was about 11 000, so that with approximately 200 eggs per female the frequency introduced was about 0.2 per cent. Subsequent records have been compiled or collected by Jones (1989, 1993*b* and this paper) and are given in table 5 and figure 5. No *bimacula* have been obtained among captures and the *medionigra* are near recessive in expression (Lees 1970; Jones 1993*b*).

(b) *Evidence of the causes of selection*

Williamson (1960) and Sheppard & Cook (1962) reviewed the evidence that there is selection acting on

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Table 3. *Numbers of typical and* medionigra *alleles recorded in samples taken from the Genetic Garden, Oxford, and maximum likelihood estimates of the selective coefficient* s *for* medionigra

The first standard error is calculated assuming the sample gives a true estimate of gene frequency, while for the second both *s* and *q* have been assumed unknown. Data from Sheppard & Cook (1962) from collections by L. M. Cook. Population estimate from Cook & Kettlewell (1960).

date	typical alleles	*medionigra* alleles	gene freq	selective coefficient	standard error	pop.
1958	61	21	0.256	—	—	
1959	118	36	0.234	0.113	0.169 or 0.218	77
1960	50	10	0.167	0.345	0.227 or 0.264	—
1961	154	26	0.144	0.158	0.179 or 0.200	—

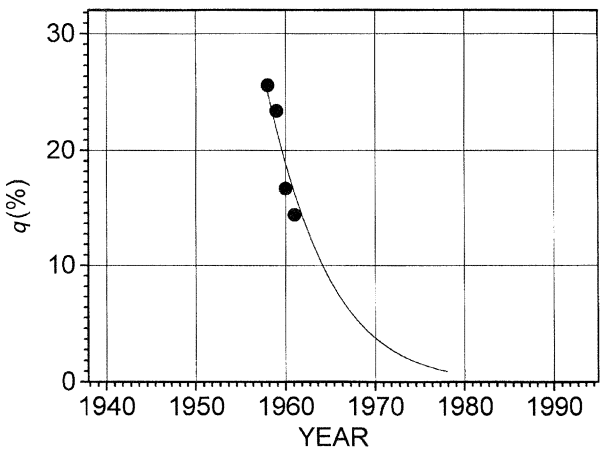

Figure 3. Frequency of *medionigra* in the artificial colony in the former Genetic garden, Oxford ($s = 0.164$). Data given in table 3.

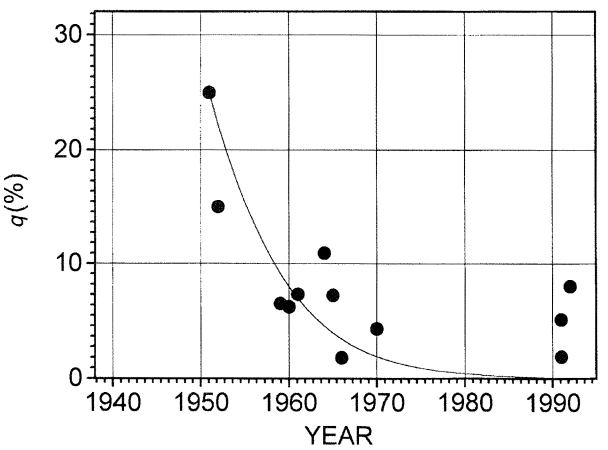

Figure 4. Frequency of *medionigra* in the artificial colony at Hinksey, near Oxford, started in 1951 at a frequency of 25 per cent *medionigra*. The curve is for an estimated 13.9 per cent disadvantage ($s = 0.139$) for the period 1951–66. Data given in table 4.

Table 4. *Genotypes scored and gene frequency estimates for the artificial colony at North Hinksey*

In all cases the broadest interpretation of *medionigra* phenotype has been used. Figures for 1964, 1965, 1966 and 1970 are from Sheppard's notebooks. The first set for 1991 is from Jones (1993*b*). The second set for 1991 and the record for 1992 are from Owen and Clarke (1993). Samples for 1952, 1959, 1960 and 1961 were taken as larvae and reared by P. M. Sheppard.

date	typical	*medionigra*	*bimacula*	gene freq
1951	4000 backcross eggs			0.25
1952	21	9	0	0.150
—				
1959	20	3	0	0.065
1960	269	32	3	0.062
1961	217	35	1	0.073
—				
1964	43	12	0	0.109
1965	66	9	1	0.072
1966	27	1	0	0.018
—				
1970	53	5	0	0.043
—				
1991	167	19	0	0.051
1991	181	7	0	0.019
1992	1007	191	0	0.080

the genotypes determined by the *medionigra* gene. Most of it comes from observations of changes in gene frequency, which are the subject of this paper. Data that most resemble a laboratory study of relative fitness come from Hinksey and Ness, where a 1:1 ratio and a 1:2:1 ratio, respectively, were expected among progeny. At Hinksey the numbers observed were 21:9 and at Ness 36:42:21. Comparison with expectation suggests a 52 per cent disadvantage of *medionigra* compared with typical at Hinksey, while at Ness both *medionigra* and *bimacula* appear to have a 42 per cent disadvantage compared with typical.

These comparisons give us no indication as to the type of selection that might be acting. So far as direct evidence goes, mutant genotypes may be less fertile than typical, and *bimacula* tends to emerge later, and is more conspicuous than the other forms (Sheppard 1953, Sheppard & Cook 1962). These effects would select against the *medionigra* gene. In addition, a study by Sheppard (1952), extended by Sheppard & Cook (1962), indicated disassortative mating when adults have a choice of mates. This would act in a frequency-dependent manner to increase the frequency of whichever allele was rare, so that in practice it would favour *medionigra*.

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Table 5. *Alleles sampled and gene frequencies in the natural colony at Sheepstead Hurst, into which* medionigra *individuals were released*

No *bimacula* individuals have been taken at this site. From Ford and Sheppard (1969), Lees (1970) and Jones (1989,1993) and unpublished data from Ford's notebooks. Mean population estimates from Ford & Sheppard (1969) and Lees (1970). Collectors as for table 2.

date	typical alleles	*medionigra* alleles	gene freq	population size
1954	eggs from *medionigra*		0.002	11000
1955	1748	2	0.001	4000
1956	870	4	0.004	4000
—				
1960	787	9	0.011	
1961	819	9	0.011	2500
1962	465	1	0.002	1500
1963	1468	14	0.009	3000
1964	1467	13	0.009	5000
1965	2306	4	0.002	7000
1966	1025	7	0.007	3000
1967	140	2	0.014	300
1968	275	1	0.004	750
1969	1043	25	0.023	3000
1970	1038	32	0.030	
1971	1009	15	0.015	
1972	585	1	0.002	
—				
1991	111	3	0.026	
1992	57	3	0.050	
1993	9	1		
1994	6	0		

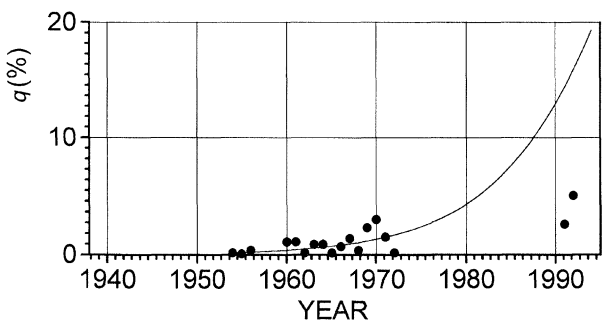

Figure 5. Frequency of *medionigra* recorded for the natural colony at Sheepstead Hurst, near Oxford, into which the *medionigra* gene was introduced in 1954 at an estimated frequency of 0.2 per cent. The curve is for a 12.7 per cent advantage ($s = -0.127$) starting from a frequency of 0.2 per cent. Data given in table 5.

(c) *Effective population size*

The population estimations shown in the tables are for the total adult emergence in a season. This is obtained by summing the daily estimates from mark recapture, interpolating values where none could be produced and dividing by the expectation of life. The resulting estimate is, of course, larger than the effective population size. Wright (1948) suggested that it might be much larger because of accidental failure of total broods. Sheppard (1951) replied that accidental loss of whole broods in the field is unlikely because the eggs are non-adhesive and are scattered widely by the females.

Another possible reason why the effective size is lower than the total estimate is that the population sometimes becomes very small. Ford (1971) and Sheppard (1951) argued that this could not account for the original rise in *medionigra* at Cothill, because there was sufficient evidence from other collectors that such a drop in numbers did not occur before the gene frequency change. Nevertheless, continued monitoring of colonies has shown that population size may be very variable, and this undoubtedly inflates the variance of gene frequency. The 46 estimates for Cothill from 1941 to 1995 (table 2) are a good illustration of the effect of population bottlenecks. The arithmetic mean is 6114, while the harmonic mean is 1281, reducing the effective number to 21 per cent of that estimated.

Variation in brood size can also affect effective number (Wright 1978). Sheppard (1951) obtained counts of eggs laid, showing that output is a function of length of life, and in a later series (Sheppard 1953) investigated variation in fertility, suggesting that *medionigra* is less fertile than typical. The large observed variance in brood size in these studies indicates that the effective number will be depressed to 81 per cent of the actual number for the first series and 65 per cent for the second (see Crow and Kimura 1970 for the calculation).

Another cause of reduction may be the courtship and mating pattern. If the numbers in the two sexes are unequal then N_e is usually calculated using Wright's expression $4N_mN_f/(N_m + N_f)$, where N_m and N_f are the numbers of males and females, respectively (Crow and Kimura 1970). The result is a figure strongly influenced by the smaller of the two. In courtship, males assemble to females, so that frequently several males may be seen round a single receptive female, and while males may mate several times, females do so only once. Since males appear to live about six days and females usually mate during the early part of their lives there is effectively an excess of males. In addition, females emerge later in the season than males, so that they may truly be rarer than males during the period when copulations can occur, and late emergers could be left without mates.

The expression given above applies if each sex may mate several times. If both sexes can mate once only, however, N_e cannot be larger than twice the number of the less common sex. If, as here, the commoner sex may mate several times, the effective number is likely to be further reduced. Whatever the pattern of success among males, their effective number must be N_f divided by the average number of matings achieved by those that mate at least once. With up to six times as many males as available females, the mean number of matings might be between 2 and 3, so that N_e would be between 1.3 and 1.5 times N_f.

Thus, what we know about factors influencing effective number suggests that it could be much lower than the estimated number. Whereas well-defined trends in gene frequency would point to strong selection, there could also be substantial fluctuations due to random effects.

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(d) *Measuring selective change*

Where the *medionigra* gene is polymorphic, the mutant form usually declines with time, suggesting that fitness is lower than that of typical. With an allele showing no dominance in expression it is a reasonable assumption that any effect of selection on the phenotype will be more marked in the homozygote than in the heterozygote. Sheppard (1951) assumed the fitness of *bimacula* to be the square of the fitness of *medionigra*, and this assumption will be used here. Although they do occur in wild-caught samples, homozygous *bimacula* individuals are rare because the frequency of the mutant gene is low, so that estimates are little affected by the precise fitness relationship chosen for the two mutant genotypes.

If p_n is the allele frequency of typical and q_n the frequency of *medionigra* in generation n and the relative fitnesses of typical, *medionigra* and *bimacula* are in the ratio $1:(1-s):(1-s)^2$ (where s is the selection coefficient), then q_{n+1} is equal to $q_n(1-s)/(1-sq_n)$. If there are A typical alleles in a sample in generation $n+1$, and B *medionigra* alleles, present either as heterozygotes or homozygotes, and $A+B=N$, then the maximum likelihood estimate of s is $(Aq_n-Bp_n)/Aq_n$. It has a variance of $NBp_n^2/A^3q_n^2$, provided q_n is known.

If q_n is unknown then it is estimated as $B/(N-As)$, with a variance of $p_n^3q_n^3(1-sq_n)(1-s)/N[p_n^3+(1-s)^3q_n^3]$. The covariance is $-(1-sq_n)^2/N$. If I_s and I_q are the second derivatives of the likelihood functions and I_w is the partial derivative of one with respect to the other, then the variance of s is $I_q/(I_w^2-I_sI_q)$. The result is that the variance of s becomes larger (see Bailey 1961).

This approach to finding s is most useful where there is a continuous series of values. For example, the Genetic Garden sampling, recorded by Sheppard & Cook (1962) produced the numbers of alleles shown in table 3. It can be seen that, although not significant, the estimated selective coefficients are substantial, between 10 and 35 per cent. They fluctuate markedly from generation to generation. Such fluctuations were noted repeatedly by Ford and Sheppard. Wright (1978) and Manly (1985) show those at Cothill to be significant if one accepts the phenotypic scoring. Models investigating the consequences of random fluctuation in gene frequency in *P. dominula* have been discussed by Wright (1948, 1978), Kimura (1954) and Tuckwell (1976).

If there is some doubt about these year-by-year changes because of difficulties of scoring, then the maximum likelihood method may still be used to produce an aggregate estimate for several successive generations by starting from a suitable value of q and finding the value of s that makes the sum of the likelihood functions zero. The standard error for this average can be obtained from the rate of convergence of the summed likelihood terms. It is appropriate if more or less constant underlying selection can be assumed. Trends will be revealed, should they exist, despite large random fluctuations due to small effective size or other causes.

4. ESTIMATES OF SELECTION

Estimates of average selection have been made for all the colonies and for different periods of years. The results are given in table 6. The curves generated have been superimposed on the data points in the illustrations of the results from different colonies.

Considering the estimates in turn, it can been seen that the Wirral Way results (figure 1) show much greater fluctuation from year to year than Cothill (figure 2). For Wirral Way, the horizontal line from the starting value of 25 per cent is the expectation if $s=0$. There are five observations above it and nine lying below. If only the values to 1976 are considered, then s is estimated as -0.1 per cent. There are gaps in the data between 1967 and 1976 and from 1977 to 1988. We have no information as to what happened during these periods, but in view of the absence of samples, this colony may have fallen to low numbers during the earlier period. If a constant progression is assumed, s is estimated as 0.8 per cent. The standard errors are large and these values do not differ from zero.

The Cothill colony produces a much more consistent pattern (figure 2). The sequences have been started arbitrarily at $q=0.1$ in 1938; moving this value slightly would make little difference to the estimated s. As it is, the estimated coefficient of 9.3 per cent for the data from 1939 to 1956 is identical to the maximum likelihood estimate obtained by Sheppard (1951) for the period 1939 to 1950. It is also very close to Wright's (1978) estimate of 10.0 per cent obtained by fitting a line to the data available from 1939 to 1968. It is difficult to believe that the sequence of frequencies observed could be due to anything other than a general disadvantage of *medionigra*, especially since some 30 generations were monitored by investigators who discussed the scoring system with each other, and the first of these (Fisher and Ford) were not looking for a trend but testing for year-to-year fluctuations. After them, more collectors were involved, and the recorded values fluctuate within a range of a few per cent. Williamson (1960, 1972) and Wright (1978) point out that the data may be divided into subsections that show somewhat different behaviour. Overall, however, the trend is down. When the full data set is used, s is found to be 7.1 per cent, not far from the first estimate. The fitted curves are falling to zero, however, whereas the data suggest that there may be an equilibrium, or equilibria, at a few per cent. This possibility is discussed by Williamson (1960, 1972), Sheppard & Cook (1962) and Ford & Sheppard (1969); it is considered in more detail below.

The four data points from the Genetic Garden (figure 3) show a consistent decline. The mean of the individual estimates of s in table 3 is 20.5 per cent. The fitted curve is for $s=16.4$ per cent, commencing with the first estimated frequency. If selection at this rate continued the frequency would have dropped to near extinction within two decades. Unfortunately the site no longer exists.

At Hinksey (table 4, figure 4) the data to 1966 show a rapid decline, with the large samples in 1991 and

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Table 6. *Selective coefficient* s *and its standard error for various colonies*

Estimates are by maximum likelihood. The *t*-value tests comparison of the observed coefficient with $s = 0$. All values except those for the Wirral Way are significant ($p = < 0.02$). Combining the smaller t values for the sites where frequency declines provides $t = 8.9$ ($p < 0.001$).

locality	starting frequency	selective coefficient	standard error	t
Wirral Way				
1961–76	0.25	−0.001	0.0327	0.03
1961–95	0.25	0.008	0.0110	0.7
Cothill				
1939–95	0.1	0.071	0.0066	10.7
1939–56	0.1	0.093	0.0094	9.9
Genetic Gdn	0.28	0.164	0.0605	2.7
Hinksey				
1951–70	0.25	0.139	0.0275	5.1
1951–92	0.25	0.060	0.0133	4.5
Sheepstead Hurst				
1955–94	0.002	−0.127	0.0231	5.5

1992, when the colony was abundant, indicating that the allele is still present. The two samples in 1991, made by different collectors, are significantly different from each other (for allele frequencies, $\chi^2 = 5.81$, $p < 0.05$). This result probably indicates a difference in scoring convention. In the large sample from 1992 there is a deviation from expectation producing a deficiency of *bimacula* individuals ($\chi^2 = 7.92$, $p < 0.01$). Selection against *medionigra* is 13.9 per cent for the early period to 1970, 6.0 per cent if the final figures are included, so that the recorded frequencies in the later samples are higher than would be expected on the basis of the calculation of selection used.

The situation at Sheepstead Hurst differs from the others in that eggs carrying the *medionigra* allele were added to an existing population. Small numbers of supposed heterozygous individuals were subsequently found (figure 5). The expression is usually very slight, and Ford & Sheppard (1969) and Lees (1970) concluded that the dominance had been changing during the course of the observations. With the possibility that some typicals may show a phenotype like a poorly expressed *medionigra* another interpretation is possible, that these individuals are in fact mis-scored typicals. Nevertheless, the condition was not noted by Sheppard before he released the gene into the colony and whatever is being scored appears to have increased in frequency. An advantage of 12.7 per cent ($s = -0.127$) is calculated from the data by the maximum likelihood method (figure 5). While it is a good fit to the main body of data, the curve diverges from the final observations. The calculated value is very sensitive to the starting frequency; if this were raised from 0.2 per cent the estimated advantage would be lowered. If the scoring is accepted, however, there is definitely selection in favour of *medionigra* in this colony. It is also apparent that different estimation procedures can produce widely differing results when the curves are extrapolated from very low frequencies.

5. GENE FREQUENCY AND TEMPERATURE

Since the expression of *medionigra* becomes more extreme in the laboratory when the pupae are maintained at high temperature, it may be asked whether the recorded frequency was higher in the field in seasons when the average temperature was high than when it was low. Owen & Clarke (1993) tested this hypothesis using gene frequencies recorded for Cothill and mean monthly air temperatures for April, May and June. They found no convincing association. We have repeated the comparison for the longer series of data now available, using mean monthly temperatures for June obtained from Manley (1974) and Parker, Legg & Folland (1992). June was chosen because that is when the insects pupate and when wing pattern is most likely to be affected by temperature. The amount of the variation accounted for, r^2, is 0.069, which is not significant ($t = 1.68$, $p = 0.1$). There is an undoubted trend in the gene frequencies, however, and a response to temperature may only be detectable after this effect has been removed. We therefore examined the regression of gene frequency on both estimated frequency and temperature. The correlation of observed with estimated gene frequency is 0.909, and is, of course highly significant ($t = 13.4$, $p \ll 0.001$). When temperature is also included, the variation accounted for is increased by 1.7 per cent, but the partial coefficient of frequency on temperature is still non-significant ($t = 1.85$, $p = 0.07$). If, like Owen & Clarke (1993), we exclude samples collected by Jones, the significance is somewhat reduced. This result simply shows that a temperature effect is not detectable. The records used, which are for central England, may not be sufficiently localized and the temperature actually experienced by the insects may be controlled to a greater or lesser extent by their choice of pupation site.

6. DISCUSSION

Gene frequency changes tend to show consistent patterns within colonies.

We suggest that the presence of these patterns shows selection to occur, despite small population size and possible mis-scoring and environmental effects. Usually there is a decline in frequency, but at Sheepstead Hurst, where the starting value was very low, there has been an apparent increase. Recent samples show similar characteristics to earlier ones. The Wirral Way population is the exception. The selective coefficient is not significantly different from zero, whereas other colonies starting from high frequencies indicate substantial selective disadvantage. Both recent samplings by the Clarkes and Sheppard's original records agree in showing wide fluctuations from year to year.

Although the results indicate fitness differences, we are no nearer knowing what type of selection might operate. The tendency for fluctuation about a low frequency at Cothill, as well as the increase at Sheepstead Hurst and the comparison of early and late figures for Hinksey all suggest that there is an

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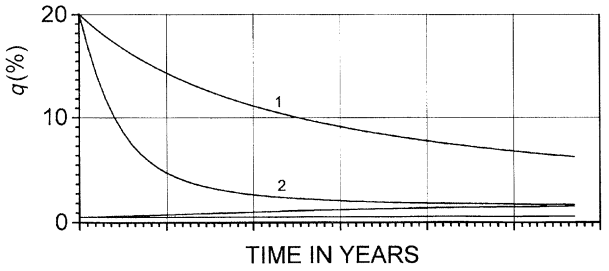

Figure 6. Curves for two models of selection moving frequency towards an equilibrium at 1.6 per cent *medionigra*. The outer pair of curves (1) are trajectories for heterozygote advantage (genotype-frequency-independent selection), where the homozygote for *medionigra* has a 25 per cent disadvantage and the typical homozygote has a 0.4 per cent disadvantage compared with the heterozygote. The inner pair (2) are for a system in which there is genotype-frequency-dependent selection. The fitnesses of the three genotypes are in the ratio $1:(1-s):(1-s)^2$, with $s = 0.2-0.3\,e^{-18.5q}$. These values were chosen so as to produce curves which would both have an equilibrium of 1.6 per cent and approach it at a rate similar to that seen in the Cothill data (figure 2).

equilibrium. Williamson (1960) investigated this possibility using Cothill data. By examining the relation of change in gene frequency to gene frequency, he showed the figures to be consistent with an equilibrium at 3.5 per cent. The rate of approach to this equilibrium point is rapid. Using the reduced major axis, Williamson found the slope of change in q on q to be -0.868. Such a value could not be achieved through selection if the selection was the net outcome of heterozygote advantage. For an equilibrium of 3.5 per cent, even assuming a fitness of zero for *bimacula*, the fitnesses of the typical and the heterozygote would have to be so close to each other that they could only drive the change at a rate of one-tenth of that observed. Taking the 39 comparisons now available, the equivalent slope is -0.584, which is significantly different from zero ($p = 0.01$). The rate of approach is still about ten times higher than it could be as a result of heterozygote advantage. The estimated equilibrium frequency is now 2.8 per cent. The plot of change in q on q is not an accurate method of obtaining the equilibrium, however, because of the range of lines which could be fitted (Williamson 1960) and the high variance. The later frequencies seem lower than 2.8 per cent (figure 2); the 24 values from 1959 have an arithmetic mean of 1.5 per cent, which is probably nearer the true equilibrium.

The alternative to heterozygote advantage is some sort of frequency-dependent selection conferring high fitness on rare genotypes, which drops off rapidly, to become a disadvantage, as genotype frequency increases. The type of effect can be modelled by assuming that instead of being constant, the coefficient s has the form $0.2 - 0.3\,e^{-bq}$. A value of $b = 18.5$ minimizes the sum of squares of deviations of the data. It provides an equilibrium of 1.6 per cent; the coefficient s is -0.1 at $q = 0$, 0.15 at $q = 0.1$, rising to 0.2 at $q = 0.5$. The rate of change is much more rapid and closer to that observed. This example is shown in figure 6, where the inner curves represent frequency-dependent selection. The outer curves show the change in gene frequency under heterozygote advantage if there were 25 per cent selection against *bimacula* and the equilibrium was 1.6 per cent.

For Hinksey and Sheepstead Hurst the assumption of constant selection cannot account for both the early change and the frequencies in the later samples. In the first case the colony remains polymorphic but the curve drops to zero, while in the second the curve rises too high. For these colonies, as well as Cothill, frequency-dependent selection towards an equilibrium at a low frequency would provide a better fit.

This exercise in model fitting shows how change in the selection pattern may affect the rate of response to perturbation, but not how the selection might be achieved. Williamson (1960) pointed out that disassortative mating, as observed experimentally by Sheppard, operates in the appropriate manner, but added that evidence available at the time did not allow a satisfactory explanatory model to be constructed. The same is true today.

The Wirral Way data appear to be an exception to the pattern observed elsewhere. In part, this may be because the population has usually been small. Figures for the size of total population have been about 100 for several generations. There are two gaps in the data, and numbers probably fell to a low level between 1967 and 1976. Considering all the evidence about effective population size, N_e is probably 75 at most. Using this value in simulations including both drift and selection, it is at once apparent that the frequency-dependent model as applied to the Cothill data does not fit. Selection of the order implied would rapidly bring the frequency to between 1 and 2 per cent, where it would remain. The observed points are compatible with the assumption that the *medionigra* allele is neutral. However, the sequence observed is also compatible with quite strong selection. With appreciable selection and a small effective population size the mode of the probability distribution of gene frequency would move towards zero with time, but the variance would be large and after the first few generations the distribution would be strongly skewed. Evaluation of the changing distribution under selection and drift is mathematically difficult (see Crow & Kimura 1970). Instead of generating the distributions, we have made repeated runs in order to find the frequency of observations which are above the initial value of 25 per cent at the end of the observed period. This frequency can then be taken as an estimate of the probability of such an observation in a single run. Ten thousand replicates have been obtained for effective population sizes of 50 and 75 respectively, with 5 per cent selection. After 34 generations the probability of a gene frequency above 25 per cent is 7.8 per cent for an effective number of 50, and 4.5 per cent for an effective number of 75. The general direction of movement and size of the yearly fluctuations in the field observations are similar to those of many of the trajectories from these simulations. When the mean squared deviation from generation to generation for the observed data is compared with the equivalent values for the simulations it is found to be significantly larger (variance ratio = 3.56 for $N = 50$

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and 5.54 for $N = 75$. With 12 and effectively infinite degrees of freedom, $p < 0.01$ in both cases). Similar variance ratios are obtained when we assume no selection instead of 5 per cent. The difference could be due to fluctuation in selection from generation to generation; alternative explanations would be that there has been mis-scoring or that the effective number is smaller than suggested.

7. CONCLUSION

Ford and Sheppard (1969 and earlier) argued that their observations indicated strong selection acting on *medionigra*, as well as fluctuation in selective coefficients from time to time and place to place. Using all the results now available, there is good consistency between different colonies, as if selection usually remains much the same in time and space. This strengthens the argument for systematic selection. The trend is evident despite small effective number and any effect of accidental mis-scoring. At the Wirral Way site there is certainly lower selection than at Cothill or Hinksey but as much as 5 per cent against *medionigra* is not excluded. It has been argued that the apparent changes in all colonies are artefacts due to mis-scoring (Owen & Clarke 1993; Owen & Goulson 1994). The risk of this is greatest at Sheepstead Hurst, where the frequency is low and the expression slight; and also in any situation where fluctuations from generation to generation are claimed as evidence of fluctuation in selection pressure. Apart from Sheepstead Hurst, the consistency observed could not arise from accidental misjudgments, especially if individuals reported to have low expression in later samples are treated as *medionigra*.

Finally, it should be emphasized that there is nothing to indicate why the gene reached a relatively high frequency at Cothill in the first place. It seems probable that the effective number in *P. dominula* colonies is often less than half the estimated population size. Despite Ford's (1971) argument for a reversal of selection, drift due to small effective population size may have contributed, as it must to the large year-by-year changes at the Wirral Way.

We are grateful to Sir Cyril and Lady Clarke and Professor W. W. Macdonald for their help and interest in this analysis, including information from Sheppard's field notes. Professors R. J. Berry, D. R. Lees, W. W. Macdonald and M. H. Williamson have provided information and comments on the typescript. G. S. Mani improved the illustrations.

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