
Long-term studies of the medionigra polymorphism in the moth Panaxia dominula: a critique

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Monitoring of the *medionigra* polymorphism in the moth, *Panaxia dominula*, at Cothill, Oxfordshire, UK, provides one of the longest and most complete data series in ecological genetics, and has provided a cornerstone in the development of this discipline. Changes in frequency between generations were used as proof of the overriding importance of selection in the prolonged and often heated selection/drift debate between R. A. Fisher, E. B. Ford and Sewall Wright. Recently evidence has emerged which suggests that the study may be flawed, particularly since phenotype appears to be influenced by environment. We offer what we regard as a proof of the inadequacy of this polymorphism for demonstrating selection, since phenotype is largely determined by temperature during the late larval or pupal phase. Hence workers at Cothill were not recording genetic processes. Assumptions that the *medionigra* form occurred only at Cothill, and that gene flow never occurred between Cothill and neighbouring populations, also appear to be untrue. The long-term study at Cothill may remain as a landmark in the history of ecological genetics only by virtue of the cautionary lessons it may teach future workers in this field.

came central to the protracted and often heated Fisher–Wright debate as to the relative importance of selection versus stochastic events in determining genetic change (this dispute is well documented by Provine, 1986). At a time when virtually no other studies of gene frequencies in natural populations were available, the Cothill study appeared to provide compelling evidence for the importance of selection, since fluctuations in frequency between successive generations were too great to be accounted for by genetic drift (although the selective agents involved, and the reasons for changes in the direction of selection from year to year, were never identified). Ford seized the opportunity to re-iterate the importance of selection, to suggest that genetic drift was of negligible importance, and to castigate Sewall Wright in a series of publications (Fisher and Ford 1947, Ford and Sheppard 1969, Ford 1964, 1975).

In 1947 Heredity published the first in a series of studies by R. A. Fisher, E. B. Ford and co-workers on the *medionigra* polymorphism in the Cothill colony of the scarlet tiger moth, *Panaxia (Callimorpha) dominula* (L.) (Lepidoptera: Arctiidae) (Fisher and Ford 1947). Studies of the frequency of morphs have continued at Cothill, Oxfordshire since 1939 (with gaps) to the present, and so represent one of the longest ecological studies ever undertaken; “No natural population of animals in the world has been so fully quantified as that of the Scarlet Tiger moth, *Panaxia dominula*, at Cothill...”, Ford (1964) (see also Jones 1989, Owen and Clarke 1993, Cook and Jones 1996). The work was initiated with a specific purpose: to provide evidence for selection acting upon gene frequencies under natural conditions. The polymorphism was interpreted as bi-allelic variation at a single locus with codominance (hence genotype BB = phenotype *dominula*, Bb = *medionigra* and bb = *bimacula*). Morph frequencies were directly translated into gene frequencies, and be-

Crucial to Ford’s interpretation of the Cothill data are three conditions (see for example Ford 1964): a) That the polymorphism be controlled by a single locus. b) That the morph frequencies can be accurately scored in successive years. c) That changes in the frequency of the *medionigra* allele at Cothill could not be due to immigration or emigration since the population was isolated and the *medionigra* allele does not occur elsewhere.

Owen and Clarke (1993) suggest that all three conditions may not hold. They point out that there are many intermediate morphs, and that classification of these is difficult. They suggest that the Cothill colony may not be isolated since there are nearby populations and males are known to fly considerable distances. They also provide limited evidence for an environmental (temperature) effect on phenotype. In 1993 we reared wild-caught late instar larvae from Cothill at a range of constant temperatures (Owen and Goulson 1994). Our data demonstrate that the frequency of the *medionigra* phenotype can be affected by temperature, casting

Table 1. Frequencies of each phenotype of *P. dominula* in wild-caught samples of adults and from wild-caught larvae reared in the laboratory at constant temperature. All samples from Axmouth, Devon except for those from East Kent. The East Kent specimens were taken between 1900 and 1950 and are held at the Natural History Museum, London. D = *dominula*, ML = *medionigra*-like, CM = classic *medionigra*.

FW spot	HW spot	24°C	18°C	Wild 1995	Wild 1996	East Kent	Classification
normal	absent	0	25	190	89	48	D
normal	yellow	0	10	6	12	20	ML
small	absent	0	0	5	13	57	ML
small	yellow	8	1	0	6	24	ML
absent	absent	0	1	0	1	1	ML
small	black	54	2	0	0	34	CM
absent	black	0	0	0	0	1	CM
normal	black	0	0	0	0	11	?
	<i>N</i>	62	39	201	121	196	

doubt on translation of phenotype frequencies to genotypes (and hence on interpretation of the Cothill data as evidence for selection).

Here we re-examine the *medionigra* polymorphism once more. We repeat our temperature experiments on larvae taken from a colony in Devon, to assess whether the *medionigra* phenotype can be induced in populations where it purportedly does not occur. We also examine the distribution of phenotypes in museum specimens from the UK (mostly pre-dating the Fisher and Ford publications). Our findings demonstrate beyond doubt that interpretation of the Cothill data as evidence for selection was incorrect.

Methods and results

Effects of temperature on phenotype frequencies

One hundred and forty-eight fourth and fifth instar larvae were collected in June 1996 from a colony at Axmouth, Devon, UK (approximately 250 km SW of Cothill). Since the eggs are scattered in flight, and the larvae are highly mobile (and not gregarious) this sample is likely to include offspring from a large number of parents. The larvae were split into two groups of equal size and placed in constant temperature cabinets at 18°C or 24°C ($\pm 0.2^\circ\text{C}$), treatments which we had previously found to produce the most marked differences in phenotype frequency (Owen and Goulson 1994). Larvae were contained within well-ventilated 30 × 15 × 10 cm plastic boxes, and fed on bramble, *Rubus fruticosus*, the usual wild food-plant at Axmouth. Pupae were removed (to prevent cannibalism by larvae), placed on damp tissue paper in identical boxes, and kept in the same temperature cabinets. Pupae were examined every day, and freshly emerged moths killed by freezing, pinned and set. They were scored for phenotype following Owen and Clarke (1993) and Owen and Goulson (1994). The classical *medionigra* phenotype differs from *dominula* in having a reduced or

absent forewing spot in the critical position and an additional black hindwing spot, and has been repeatedly illustrated (e.g. Fisher and Ford 1947, Clarke et al. 1990, 1991). Intermediate phenotypes occur with either a yellow or absent hindwing spot and/or a reduced or absent forewing spot, and would probably have been scored as *medionigra* in the past (Owen and Clarke 1993). We classify such phenotypes as *medionigra*-like (Owen and Clarke 1993 describe and illustrate the range of phenotypes). For comparison, in July 1995 and 1996 wild moths from the same site were caught, marked, scored for phenotype and released. To maintain consistency, all scoring was carried out by one person (DFO).

Manipulation of temperature during the final instar and during the duration of the pupal phase resulted in a dramatic change in frequencies of phenotypes broadly in accordance with our previous work (Owen and Goulson 1994) (Table 1). Rearing at a constant 18°C produced frequencies approximating to those in the wild-caught sample ($\chi^2_1 = 1.30$, n.s., pooling *medionigra* and *medionigra*-like) (Fig. 1). In contrast, rearing at a constant 24°C produced a substantial majority of classic *medionigra*, a phenotype absent among the 121 wild-caught specimens from 1996 ($\chi^2_2 = 154.5$, $p < 0.001$) (and also absent among 201 wild specimens sampled in 1995) (Fig. 1). Not a single *dominula* was present in the 24°C treatment. Although the 18°C treatment produced a small number of classic *medionigra*, the frequencies differed markedly from the 24°C treatment ($\chi^2_2 = 72.3$, $p < 0.001$). Interestingly, there was a significant shift in frequencies among wild-caught specimens between 1995 and 1996, with an increase in the proportion of *medionigra*-like phenotypes ($\chi^2_1 = 28.5$, $p < 0.001$).

Distribution of phenotypes in the UK

To assess the distribution of classic *medionigra* and *medionigra*-like phenotypes in the UK we examined the

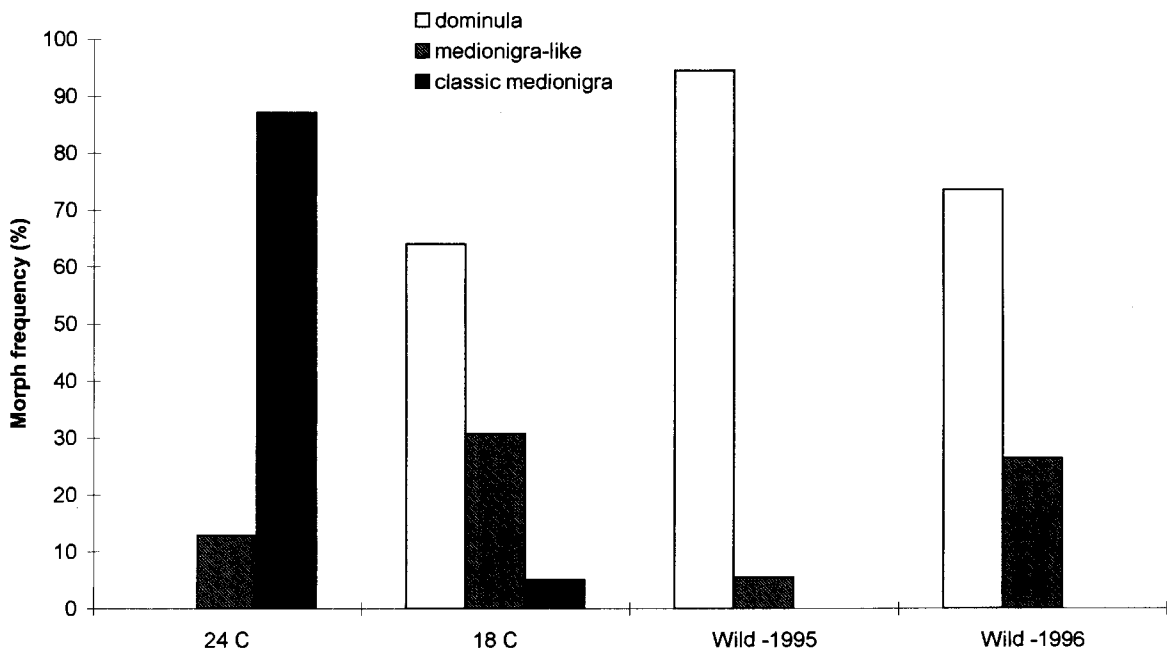


Fig. 1. Morph frequencies among *P. dominula* caught as adults in 1995 and 1996, and frequencies among laboratory reared larvae caught in 1996 and reared at either 18°C or 24°C. All samples originated from Axmouth, Devon. Sample sizes are given in Table 1.

extensive collection of *P. dominula* in the Natural History Museum, London. Five hundred and four specimens from Kent, Wiltshire, Hampshire, Dorset, Devon, Camarthenshire, Glamorgan and Pembrokeshire were scored for phenotype as above.

These specimens demonstrate that classic *medionigra* and *medionigra*-like phenotypes occur at sites other than Cothill (Table 2). It would be naive to expect these specimens to represent an unbiased estimate of actual frequencies in the wild since unusual specimens are likely to have been oversampled, but nonetheless it is obvious that *medionigra*-like and classic *medionigra* phenotypes are to be found scattered throughout the range of *P. dominula* in England (the Welsh samples are too small to indicate the presence or absence of such morphs). Our wild-caught samples and specimens resulting from temperature experiments include a range of intermediate forms; a breakdown of the specimens from East Kent indicates that these forms have occurred in wild specimens for many years (Table 1).

Discussion

“The polymorphism of *P. dominula* at Cothill is controlled by a gene whose effects can be detected in all three genotypes; consequently its frequency is susceptible of direct calculation” (Ford 1964).

Our rearing experiments establish beyond doubt that temperature can substantially alter phenotype frequen-

cies in *P. dominula*, and demonstrate that the *medionigra* phenotype can be induced in a population far from the Cothill colony. The magnitude of the temperature response (Fig. 1) calls into doubt whether the *medionigra* polymorphism has any substantial genetic basis. Ford states throughout his work that phenotype and genotype are directly related, but the evidence for this assumption is scant, and temperature effects upon phenotype were known (Kettlewell 1943/44). Ford based his study on a single cross reported second-hand by Cockayne (1928). The cross was purportedly between two *medionigra* (heterozygote) parents, but at the time one parent was scored as *dominula*. Only when the offspring produced an 1:2:1 ratio was this parent reclassified as *medionigra* (after both parents had been discarded). Ford and co-workers P. M. Sheppard and H. B. D. Kettlewell undoubtedly bred many *P. dominula* over the years, but if they repeated this cross they did not publish the results and as far as we can ascertain no specimens from known crosses remain (for further discussion of evidence of genetic controls see Owen and Goulson 1994).

Further evidence for the importance of temperature on phenotype frequencies was provided by Owen and Clarke (1993) who found a significant correlation between frequency of the *medionigra* form at Cothill and mean temperatures for April. However, in April the larvae are rather younger than those used in this study, and no correlation was found with May or June temperatures (which correspond to the larval and pupal

Table 2. The distribution of occurrence of phenotypes of *P. dominula* based on specimens held at the Natural History Museum, London. Most specimens were collected between 1900 and 1950.

Locality	<i>dominula</i>	<i>medionigra</i> -like	classic <i>medionigra</i>	<i>N</i>
Kent	+	+	+	196
Wiltshire	+	+	+	90
Hampshire	+	+	+	187
Dorset	+	+	—	13
Devon	+	+	—	18
Camarthenshire	+	—	—	14
Glamorgan	+	—	—	1
Pembrokeshire	+	—	—	4

stages studied here). Since we do not know precisely which aspects of temperature affect phenotype, and the duration for which immature stages are sensitive, these data are hard to interpret. The critical factor may be, for example, the minimum or maximum temperature during just one or two days, or even the magnitude of temperature changes during these days, aspects of climate which are unlikely to relate closely to mean monthly temperatures.

Although we cannot rule out some genetic contribution, we conclude that the *medionigra* polymorphism is determined predominantly by temperature effects upon late-larval or pupal development. We also question whether it is appropriate to describe this as a polymorphism since variation is continuous; a range of intermediates occur in both wild-caught and laboratory reared samples.

“...Cothill is the only place where *Panaxia dominula* is known to be polymorphic for the *medionigra* gene...” (Ford 1964).

Our temperature experiments demonstrate that moths taken from sites far from Cothill have the potential to become *medionigra*; indeed under the right environmental conditions *all* individuals became classic *medionigra* or *medionigra*-like. Specimens at the Natural History Museum show that *medionigra* and *medionigra*-like individuals actually occurred throughout the range of *P. dominula* in England, at some sites (such as East Kent) in considerable numbers. Many of these specimens were collected by co-workers of Ford, including H. B. D. Kettlewell. It is thus surprising that Ford chose to dismiss them, stating that “single specimens of what appear to be *medionigra* have been found as rare aberrations in other places” (Ford 1964). To our knowledge no attempt was made to quantify the frequency of these “rare aberrations” in any natural population of *P. dominula* other than that at Cothill. One could argue that *medionigra* forms induced in the Axmouth population are phenocopies of a genotype found only at Cothill, or at the very least that alleles present only at Cothill render individuals there more susceptible to temperature effects. However, the most parsimonious explanation is that the frequency of *medionigra* forms is largely determined by environment in all populations;

there is no convincing evidence for a genetic distinction between the Cothill population and others.

“...it [*P. dominula*] rarely wanders even a few hundred yards from its habitat, so that the Cothill and Sheepstead Hurst colonies in Berkshire are completely isolated from one another, though but one and a quarter miles apart” (Ford 1964).

P. dominula has been reported at light and assembled to virgin females at a distance of 0.5 km from known colonies (Owen and Clarke 1993). It is found throughout the Thames valley in Oxfordshire (Owen and Clarke 1993). The favoured foodplant, *Symphytum officinale*, is abundant along the small stream running from Cothill to Sheepstead Hurst. In 1993 (a year of high population density at Cothill), we found larvae on *Symphytum* almost all the way along the stream (Owen 1996). There is no reason to suppose that it has not been like this for many years. Cothill and Sheepstead Hurst are not “completely isolated from one another”.

Conclusions

Previously we stated that *P. dominula* was a poor choice upon which to base a strong pro-selection argument, but we accepted that there was probably a genetic basis to the *medionigra* phenotype albeit modified by environment (Owen and Clarke 1993, Owen and Goulson 1994). In the light of our new studies we suggest that the *medionigra* phenotype is predominantly, perhaps entirely, a product of environment. The studies of Ford and co-workers upon *P. dominula* which featured so prominently in the Fisher–Wright debate and which were at the forefront of research into ecological genetics in the 1950s and 1960s are fatally flawed. Sadly, we must thus infer that the principal conclusion from this, the longest study in ecological genetics ever to be undertaken, was incorrect and tells us little or nothing about the role of selection or drift. Ironically, by apparently demonstrating that selection could be studied in natural populations, this work spawned a host of subsequent and more revealing studies upon other species.

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