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Effect of temperature on the expression of the medionigra phenotype of the moth Panaxia dominula (Lepidoptera: Arctiidae)

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The well-known *medionigra* polymorphism in the scarlet tiger moth, *Panaxia dominula*, occupies an unique position in the history of ecological genetics. Year to year changes in phenotype frequency at the Cothill (Oxfordshire) colony were too great to be explained by random genetic drift, and were used as evidence for selection in a prolonged and heated debate between, on one side, E. B. Ford and R. A. Fisher, and on the other, Sewall Wright. Here we report experiments which clearly demonstrate that expression at the *medionigra* locus is largely determined by temperature. Since the genotype is not necessarily recognisable from the phenotype, the long series of field scored samples from Cothill do not accurately record changes in the *medionigra* allele frequency from year to year. Hence interpretation of the data as evidence for selection is invalid.

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In a critical re-assessment of the well-known and much quoted *medionigra* polymorphism in the scarlet tiger moth, *Panaxia dominula*, (L.), Owen and Clarke (1993) predict that temperature could affect expression of the *medionigra* phenotype. If this is so, the results of monitoring the *medionigra* allele frequency at the famous colony at Cothill, Oxfordshire, England, would be largely invalidated. This colony, which has been studied since 1939, was central to the Fisher-Wright debate on the relative importance of natural selection and genetic drift in maintaining and changing allele frequencies in natural populations (Jones 1989, Owen and Clarke 1993). Fisher and Ford considered the data from Cothill as providing powerful evidence for selection as the primary agent responsible for maintaining and changing allele frequencies, and extrapolated from this example to suggest that stochastic processes were of minimal importance (Fisher and Ford 1947, Ford and Sheppard 1969, Ford 1975). Here we report on the results of temperature experiments conducted in May-July 1993 which cast further doubt on the usefulness of the Cothill data in differentiating the effects of selection and drift.

Methods

Final instar larvae were collected in May 1993 at Cothill (75 larvae) and at North Hinksey (185 larvae), a colony artificially established in 1951 5 km from Cothill. They were placed in constant temperature cabinets at 12°, 18° and 24° (± 1°C) in well-ventilated 30 × 15 × 10 cm plastic boxes, and fed on comfrey, *Symphytum officinale*, the main larval foodplant in Oxfordshire. 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 two days, and freshly emerged moths killed by freezing,

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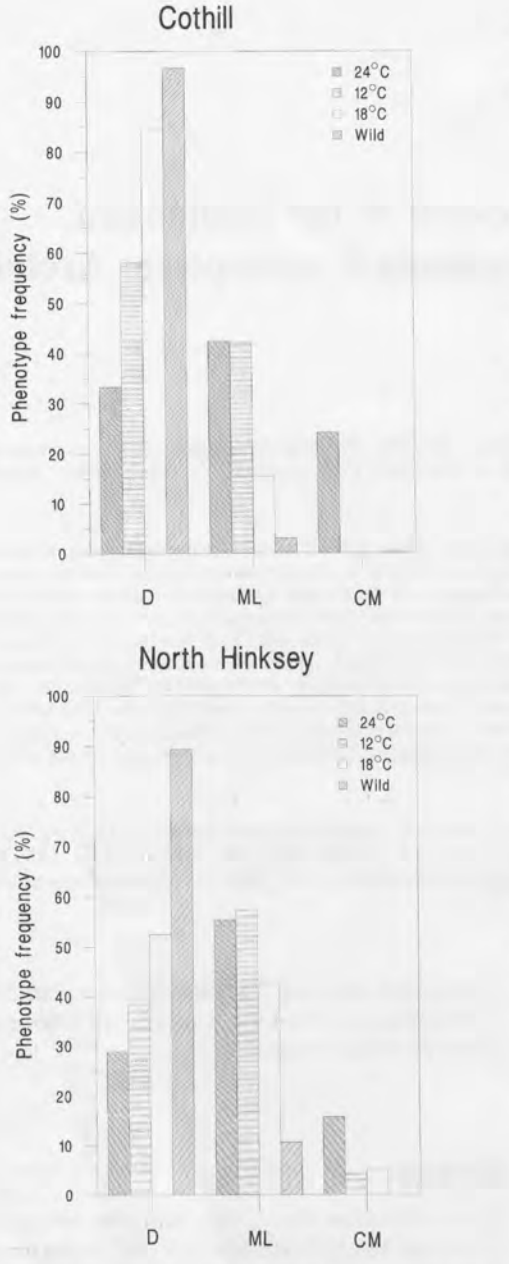

Fig. 1. Phenotype frequencies of *P. dominula* following rearing wild collected fifth instar larvae at three different constant temperatures (12°, 18° and 24°C (± 1°C)), compared to wild samples of adults from the same cohort. D = *dominula*, ML = *medionigra*-like, and CM = classical *medionigra*. Sample sizes were, for Cothill 19, 13, 33 and 300, and for North Hinksey 47, 40, 83 and 66 (12°, 18°, 24°C and wild caught adults, respectively).

pinned and set. They were scored for phenotype in exactly the same way as described in Owen and Clarke (1993), separating *dominula* (D), classical *medionigra* (CM) and intermediate phenotypes which would probably have been scored as *medionigra* in the past (*medionigra*-like, ML). For comparison, in July 1993 wild moths from both sites were caught, marked, scored and released. To maintain consistency, all scoring was carried out by one person (D.F.O.).

Results

The frequency of phenotypes differs markedly according to the temperature regime under which the immature stages are reared, and particularly between insects reared at a constant temperature compared to wild samples of the same cohort (Fig. 1). *Medionigra* allele frequencies, calculated by considering both classical *medionigra* (CM) and *medionigra*-like (ML) as heterozygotes (as both would probably have been scored as such in the past) vary from 1.8% (Cothill wild sample) to 45.5% (Cothill 24°C). The two sites differ significantly ($\chi^2_{(1)}$ = 101, $p < 0.001$), with the frequency of *medionigra* phenotypes generally higher at North Hinksey than at Cothill. In the North Hinksey samples, as at Cothill, the allele frequency is lowest in the wild sample (5.3%), and highest when reared at 24°C (43.4%). The treatments have a consistent effect: apparent frequency of the *medionigra* allele increases according to treatment in the order: wild sample < 18°C < 12°C < 24°C. Ten of the 12 possible pairwise comparisons reveal a significant difference between treatments (Table 1).

The principle cause of deaths in laboratory-reared larvae was parasitisation by *Carcelia lucorum* (Tachinidae: Diptera), which killed 4.6% of larvae. These larvae were parasitised prior to capture (the parasite overwinters in the host, T. H. Ford, pers. comm.), and thus would presumably have died whatever temperature they experienced. Mortality due to other causes was minimal (< 1%), with the exception of pupae in the 12°C treatment which exhibited a reduced emergence rate (12% failed to emerge). Hence differential mortality does not explain differences between temperature treatments, and massive selection against *medionigra* phenotypes in the wild would be necessary to produce the comparatively low frequencies of *medionigra* in the wild samples.

Discussion

Our results strongly suggest that expression of the *medionigra* allele is mediated to a substantial extent by temperatures experienced by the late larval or pupal stages. This should be no surprise, for wing pattern is known to be sensitive to temperature during a short, critical phase of pupal development in Lepidoptera (Nijhout 1991). Interestingly, the experimentals responded to the 12°C treatment, even though this is about the same as the mean air temperature in the wild; however, air temperature fluctuates markedly, unlike the constant temperatures ex-

Table 1. Variance analyses comparing mean phenotype frequencies for wild and experimentally reared samples of *P. dominula*. Calculations based on *dominula* versus classical *medionigra* and *medionigra*-like combined. MS = mean square, df = degrees of freedom, F is the variance ratio; * 0.05 > P > 0.01, ** 0.01 > P > 0.001, *** P < 0.001.

	Cothill			North Hinksey		
	MS	df	F	MS	df	F
Treatments	16.29	3	89.30***	17.37	3	23.22***
Residual	0.18	361	–	0.75	232	–
Wild vs 12°C	3.31	1	18.14***	21.94	1	29.33***
Wild vs 18°C	0.15	1	–	6.08	1	8.12**
Wild vs 24°C	47.18	1	258.64***	48.36	1	64.66***
12°C vs 18°C	0.79	1	4.33**	3.46	1	4.62*
12°C vs 24°C	8.29	1	45.47***	1.92	1	2.57
18°C vs 24°C	12.32	1	67.52***	11.51	1	15.39***

perienced by experimental larvae. Our results perhaps suggest that all that is required to produce a *medionigra* phenotype is either exposure to a high temperature or to an equable temperature regime.

From the historical viewpoint, our most telling results are from the Cothill material. The frequency of ML + CM for the three treatments combined is 49.2%, which gives an allele frequency of 24.6%, more than twice the all time high recorded in the wild since the start of the project in 1939 (11.1% in 1940, Sheppard and Cook 1962). The North Hinksey colony was started in 1951 with 4 000 eggs from backcross *dominula* × *medionigra* matings and so the initial frequency should have been about 25% (Sheppard and Cook 1962). It was expected that the frequency would fall, and indeed samples taken in 1952 and 1959–61 demonstrated a fall and strong adverse selection was invoked (Sheppard and Cook 1962). However, the combined ML + CM frequency in our temperature experiments is 63.6%, giving an allele frequency of 31.8%, conspicuously higher than the presumed 25% in the founders.

We suggest that the *medionigra* allele is far more common than field scoring would suggest, and that a substantial but unknown number of phenotypes scored each year as *dominula* were genetic *medionigra*. It is not difficult to envisage what happens from year to year: May and June air temperatures vary and not all larvae are equally exposed to the same temperature (the habitat is heterogeneous). Thus it is likely that the frequency of phenotypically identifiable *medionigra* depends much more on the vagaries of the weather than on the action of natural selection. Hence reported changes in gene frequency may represent nothing of the sort, but rather chance exposure of larvae to higher or lower temperatures than usual, perhaps for only a brief period.

Indeed, there is evidence that from the very first difficulties were encountered in accurately recording genotypes: the original and frequently-quoted cross which formed the genetic basis for the long-term field study was reported by Cockayne (1928) as between two *medionigra* (heterozygote) parents, producing the expected 1:2:1 phenotypic ratio in the offspring. Yet at the time of the cross, one of the parents was scored as *dominula*. Only with hindsight, and after the parents had been discarded, was it decided that both parents must have been *medionigra*, purely because of the offspring ratio. Ford and co-workers never attempted to repeat the cross, or left no record if they did. Indeed, to our knowledge the genetics of the polymorphism was never convincingly verified, although limited breeding results by others tend to confirm the single pair of alleles hypothesis (Asbrook 1939, Symes 1953a, b, 1954, Clarke et al. 1991). We recently examined the offspring of a *dominula* (albeit a rather unusually coloured one) × *bimacula* mating obtained by H. B. Williams in 1947. The specimens, in the Natural History Museum, London, comprise 70 CM and 9 ML, but the range of variation (they should all be heterozygotes) is continuous from *dominula*-like ML to *bimacula*-like CM; indeed at least three could easily be classified as *bimacula*.

If we retain the assumption that the *medionigra* polymorphism is controlled by a single locus, and if, as we propose, the frequency of the *medionigra* allele is far higher than field scoring suggests, then we must question why the homozygote *bimacula* has not been recorded at Cothill since 1959. One possibility is that the fitness of *bimacula* is so low that it appears only as a rarity. We consider it more likely that *bimacula* is also subject to variable expression, and that as a consequence genetic *bimacula* may not be recognised as such. In temperature experiments we found individuals with a *bimacula* forewing, but a *medionigra* hindwing, and the extensive collection of *P. dominula* in the Natural History Museum, shows that there is a complete gradation from *medionigra* to *bimacula*.

In the absence of evidence to the contrary we accept the single allele hypothesis as the most simple explanation for the *medionigra* polymorphism, but note that the heterozygote in particular is frequently not recognisable as its phenotype is subject to considerable modification by temperature and grades continuously with the phenotypes of both homozygotes. This means that the entire run of results from Cothill cannot be accepted as evidence that either natural selection or genetic drift maintain and

alter allele frequencies. As indicated in the earlier paper (Owen and Clarke 1993), in retrospect the moth was a poor choice upon which to base a strong pro-selection and anti-drift argument: the Cothill scarlet tiger project, one of the longest running in the history of ecological genetics, must be judged as a valuable contribution in terms of the research techniques developed, but as a failure in terms of its contribution to an understanding of the operation of natural selection, claims to the contrary notwithstanding.

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