Recent History of Melanism in American Peppered Moths

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Industrial melanism in peppered moths has been studied most intensively in Britain. The first melanic phenotype (effectively solid black) was recorded near Manchester in 1848. By 1895 about 98% of the specimens near Manchester were melanic, and this once rare phenotype had spread across regions of the country blackened by industrial soot. In rural, unpolluted regions, well away from industrial centers, the pale phenotype (peppered with white and black scales) remained the predominant form. During the latter half of the 20th century, following legislation designed to improve air quality, melanics began to decline in frequency and are now rare where once they had been common. Similar evolutionary changes have occurred elsewhere, but records from outside Britain are fragmentary. We have extended previous surveys of American peppered moth populations and present a composite picture of the recent decline in melanism in northern industrial states—Michigan and Pennsylvania—where melanic phenotypes decreased from more than 90% in 1959 to 6% by 2001. We contrast these changes to the near absence of melanism in a southern state—Virginia—during that same period. As in Britain, the decline in melanism in American peppered moths followed clean air legislation.

Industrial melanism refers to the evolution of darkened body coloration in animal species that inhabit environments blackened by industrial pollution. Although the phenomenon has been well documented in many lepidopteran species, the peppered moth [*Biston betularia* (Geometridae)] has been the most intensively studied example. For recent reviews see Cook (2000), Grant (1999), Grant and Clarke (2000), and Majerus (1998).

In the United Kingdom the melanic phenotype (f. *carbonaria*) nearly replaced the pale form (*typica*) in vicinities affected by coal-fired industries (Kettlewell 1973), but in recent decades, following government regulations designed to improve air quality, melanic phenotypes have declined sharply in frequency (Clarke et al. 1985, 1994; Cook et al. 1999; Cook and Grant 2000; Grant et al. 1996, 1998; Mani and Majerus 1993; West 1994).

Intermediate phenotypes (f. *insularia*) also became common in industrial regions of Europe and in some parts of Britain (see Kettlewell 1973) and have recently declined (Brakefield and Liebert 2000; Cook and Grant 2000). The dynamics of frequency changes of the intermediate phenotypes, however, are complicated by dominance relationships among multiple alleles as their frequencies change under directional selection [see Cook and Grant (2000) for analysis], but overall the trend has been the reduction of all nontypical alleles.

In North America (Michigan), increases (Owen 1961, 1962a,b) and decreases (Grant et al. 1995, 1996, 1998) in melanism in the subspecies *B. betularia cognataria* have paralleled the changes most thoroughly documented in Britain. But unlike Britain, the historical record of melanism in America is fragmentary. A 32-year gap in the record separates the early and late samples taken in Michigan. Yet during those "middle" years when sampling in Michigan was abandoned, peppered moth populations elsewhere were surveyed for melanism. For example, Manley (1988) reported intermediate frequencies of melanic peppered moths in Pennsylvania from 1971 through 1986. In this report we show, through continued sampling in both regions, that Pennsylvania and Michigan have arrived at the same point. Based on our data and those previously published by other authors, we present a composite picture of the decline in melanism in American peppered moths.

From the Department of Biology, College of William and Mary, Williamsburg, VA 23187-8795. We thank Jillian Bechberger, Cathy Grant, and Louise Lesevich for assisting with field collections, the staff at Bays Mountain Park in Kingsport, TN, for facilitating our moth trapping there, Ron Nussbaum for arranging our return to Michigan's George Reserve, and Carolyn Stevens for providing air quality data for Virginia. We are especially indebted to David West for donating his data from Virginia's Mountain Lake Biological Station. Two anonymous reviewers helped us improve the manuscript. We dedicate this article to the memory of our esteemed colleague and close friend, Denis F. Owen, the pioneer of industrial melanism studies in America. Address correspondence to B. S. Grant at the address above or e-mail: Geometrid@aol.com.

Table 1. The frequencies of melanic *B. betularia cognataria* in samples from natural populations in different regions of the United States from 1994 through 2001

Location	Date	Melanic (%)	*N*	Data source
Pinckney, MI	1994	16.0	25	Grant et al. 1995
	1995	20.0	35	Grant et al. 1996
	2001	5.3	283	This report
Eastern PA	1996	17.5	137	Grant et al. 1998
	1997	9.7	549	Grant et al. 1998
	1998	9.5	714	This report
	1999	11.6	777	This report
	2000	10.0	40	This report
	2001	6.8	177	This report
Weirton, WV	1999	6.0	301	This report
	2000	3.0	36	This report
Pembroke, VA	1994	0.0	118	West DA, personal communication
Tazewell, VA	1997	0.0	135	Grant et al. 1998
Kingsport, TN	1998	0.0	138	This report
	1999	0.0	186	This report

Materials and Methods

During the summers of 1998–2001 we sampled populations of *B. betularia cognataria* at the following locations: Livingston County, Michigan; Columbia, Luzerne, and Schuylkill Counties, Pennsylvania; Sullivan County, Tennessee; Hancock County, West Virginia. We collected moth samples by using mercury-vapor light traps and assembling traps [for details about trap designs see Grant et al. (1996, 1998)]. Each peppered moth in the samples was identified by phenotype as melanic (solid black), typical (patterned wings), or intermediate (*insularia*). The range of phenotypic differences results from multiple alleles at a single locus, with the allele producing full melanism as dominant (Lees and Creed 1977; West 1977).

The phenotypic distinction between full melanics and typicals is obvious even to inexperienced workers, but intermediates vary widely in appearance and scoring of the extremes is subjective. Some reports on American populations list intermediates (Owen 1962a; West 1977), whereas others do not (Manley 1988; Sargent 1974; Treat 1979). For consistency in comparing our data to the published reports from various authors, we assigned our specimens to two categories: melanic and nonmelanic (typicals plus intermediates). The sex of each moth collected was also recorded, but as females comprise a very small fraction of samples taken by light traps, and none by assembling traps, distinctions about gender are not included here.

Results and Discussion

The results from our population surveys are listed in Table 1. We include results of recent surveys from the same general areas for comparison.

Michigan

Michigan interested us most for historical reasons. From 1959 through 1962, D. F. Owen (Grant et al. 1996; Owen 1962a) trapped 598 peppered moths at the George Reserve (a biological field station near Pinckney operated by the University of Michigan); of that number, 533 of the specimens were solid black (about 90%). By the time the George Reserve was revisited in 1994 and 1995, the percentage of melanic specimens had dropped to 18.3% (Grant et al. 1996). Even now the decline continues. Our present (2001) sample of 283 moths included only 15 melanics (5.3%). The difference between the 2001 sample and the 1994–1995 sample is statistically significant ($\chi^2 = 12.00$, $P < .001$).

Pennsylvania

Although the earliest records of melanic peppered moths in North America date from 1906 in Pennsylvania (Owen 1961), population surveys reporting melanic frequencies did not begin until the 1970s (Manley 1988). Manley's survey spanned 16 years, ending in 1986. He showed a decline in melanism in Schuylkill County from more than 50% to 38%. In 1996 and 1997 Grant et al. (1998) surveyed moth populations in the adjoining counties of Columbia and Luzerne. Their study sites included a coal mining region near Wilkes-Barre with a highly disturbed terrain surrounded by culm banks, a nearby (13 km) rural, mountainous setting (Back Mountain), and a farmland community near Catawissa, 60 km southwest of Wilkes-Barre. Although the melanic frequencies declined significantly between years, there were no significant differences among these locations within years. We have continued to sample the same two counties since 1998, and in 2001 we included Schuylkill County at Hegins (11 km southeast of Manley's original site reported as 11 km northeast of Klingerstown). Our 2001 Schuylkill sample (1 melanic, 53 nonmelanic) does not differ significantly from our 2001 Luzerne/Columbia sample (11 melanic, 112 nonmelanic) ($\chi^2 = 2.99$, $P \leq .1$). In Table 1 we have pooled the Pennsylvania subsamples as homogeneous within years to simplify the analysis of the changes over years for the general region. The decline in the frequency of melanics over the 6 years of our survey (1996–2001) is significant by regression ($b = -0.30$, $t = -4.71$, $P < .01$, $R^2 = 0.79$).

Michigan and Pennsylvania

Here we attempt to piece together a composite picture of the decline in melanism for the northern industrial states represented by Michigan and Pennsylvania. The difference in the distribution of melanic and nonmelanic phenotypes between the 1994–1995 Michigan sample (18.3%) and the 1996 Pennsylvania sample (17.5%) is not significant ($\chi^2 = 0.02$, $P > .75$), nor is the difference between the concurrent 2001 samples taken in Michigan (5.3%) and Pennsylvania (6.8%) significant ($\chi^2 = 0.43$, $P > .50$). We therefore argue that the significant declines in melanism we have observed in both states have occurred in close parallel over the past 8 years. Whether we can extend that same argument back through the middle years (using Manley's data for Pennsylvania to splice in the missing years for Michigan) is a matter of conjecture. What is clear is that melanism has declined very significantly over the past several decades in both Michigan and Pennsylvania. In Figure 1 we have plotted this history.

Other Regions

Melanic peppered moths have been reported across the width of continental North America (see Rindge 1975), but except for Michigan and Pennsylvania, information about frequencies is absent or the frequencies reported have been low. In central Massachusetts, Sargent (1974) reported 6 melanics among 135 specimens (4.4%) caught over a 3-year period (1971–1973), and Treat (1979) reported an average frequency of 7.3% melanics in western Massachusetts from 1958 through 1977. Unfortunately Treat's sample sizes were too small after 1967 to calculate frequencies with confidence, and in 6 of the last 9

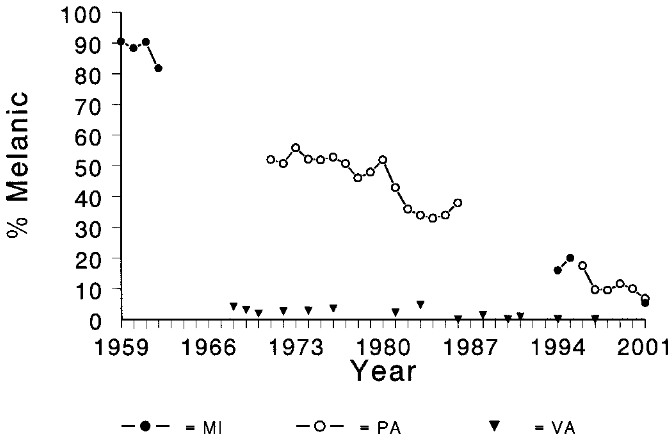

Figure 1. The decline in melanism in American peppered moths in the northern states—Michigan (solid circles) and Pennsylvania (open circles)—and the relative absence of melanism in a southern state—Virginia (inverted triangles). Early Michigan samples (1959–1962) from D. F. Owen, middle Pennsylvania samples (1971–1986) from T. R. Manley, and Virginia samples primarily from D. A. West. See text for references to previously published data.

years in which moths were caught, no melanics were among them. We examined 18 pinned specimens from Massachusetts deposited from 1966 through 1978 in Harvard University's Museum of Comparative Zoology; all were typicals. Our own limited trapping efforts in New England in 1989 likewise yielded no melanics.

A relatively long-term record exists for Virginia. West (1977) reported low frequencies of melanic peppered moths in the southern Appalachians and at the Mountain Lake Biological Station near Pembroke, where he has monitored a steady decline in melanism from 4.2% in 1968 to zero (0.0%) in 1994 (West DA, personal communication). Including our own samples with West's, that site has been sampled 13 times over a 26-year period, with an average annual sample size of 235 $\pm$ 64. For comparison with Michigan and Pennsylvania, we have plotted the melanic frequencies for Virginia in Figure 1.

In 1997 Grant et al. (1998) moved trapping westward to Tazewell, Virginia, and Gary, West Virginia. No melanics were among their samples (N = 135). In 1998 and 1999 we ran traps at Bays Mountain Park in Kingsport, TN, and again we failed to find any melanics (N = 324 over 2 years). It appears that melanic peppered moths are now absent or at a very low frequency in rural western Virginia and urban eastern Tennessee.

In 1999 and 2000 we ran traps in the northern panhandle of West Virginia at Tomlinson Run State Park, near Weirton, a steel-producing city west of Pittsburgh. Of the 337 peppered moths caught over the 2 years, 19 were melanic (5.6%) (Table 1). Unfortunately the history of melanism at that location is unknown, but it does provide us with an approximate geographic midpoint between our collection sites in southeastern Michigan and northeastern Pennsylvania. Indeed, had we not found melanism at Weirton we would have been surprised.

Common Causes

The changes in the percentages of the phenotypes comprising peppered moth populations reflect changes in allele frequencies. Change in allele frequency is the very definition of evolution. Of the evolutionary forces (mutation, gene flow, drift, and selection), only selection can explain the steady direction, velocity, and magnitude of the allele frequency changes that have been observed in peppered moth populations in Britain, America, and Europe.

The proximal mechanism by which the selection occurs is not without controversy (Sargent et al. 1998), but only differential predation by birds on moths that are variously conspicuous against backgrounds of different reflectance has been repeatedly supported by experiment (reviewed by Cook 2000; Grant 1999; Grant and Clarke 2000; Majerus 1998). Because reflectance from the surface of tree bark is strongly negatively correlated with atmospheric levels of suspended particles (Creed et al. 1973), the testable prediction is that melanic phenotypes should be more common in sooty, polluted regions than they are in unpolluted regions. This prediction was clearly supported in Britain by the incidence of melanism among peppered moth populations surveyed across the country during the 1950s (Kettlewell 1973), with "frequency patterns over a mosaic environment smoothed by migration" (Cook 2000).

Several studies have shown that the geographic distribution of melanism in British peppered moths is more strongly correlated with sulfur dioxide (SO_2) concentration than with smoke (e.g., Lees et al. 1973; Steward 1977). SO_2, as a gas, is more widely dispersed in the atmosphere than particulate matter that tends to settle locally as soot (Cook et al. 1999). Although selection may operate locally, gene flow has contributed to more gradual clines in melanism in peppered moths, a relatively mobile species, than in the sedentary species *Gonodontis* (= *Odontoptera*) *bidentata,* in which the frequency distribution of melanic phenotypes is more sharply subdivided over its range (Bishop et al. 1978; reviewed by Lees 1981; Majerus 1998).

In 1956 Britain initiated the Clean Air Acts to establish so-called smokeless zones in heavily polluted regions. Following significant reductions in atmospheric pollution, melanic peppered moths declined in frequency as the typical form recovered (Clarke et al. 1985). The predicted correlation between changes in the levels of pollution and the incidence of melanism has been firmly established in Britain.

The coincidence of a significant reduction in atmospheric pollution and a sharp decline in melanism has also occurred in American peppered moths (Grant et al. 1996). In Figure 2 we compare the SO_2 concentrations in southeastern Michigan, where melanism has declined from 90% to 5% (Figure 1), and western Virginia, where melanism has not exceeded 5% over the same time interval (Figure 1). For each region we attempted to secure long-term annual records for SO_2 concentration closest to our moth trapping sites. While the distances are about the same (65–75 km east of the trapping sites), the SO_2 data are not directly comparable for several reasons: the procedures used in assessing SO_2 concentrations have changed three times over the course of monitoring, using manually performed gas-bubbler assays in the early years compared to continuous monitoring by machines using ultraviolet (UV) fluorescence in recent years; the locations of monitoring stations within regions have been moved several times; and the scales have changed from $\mu g/m^3$ to ppm, necessitating approximations for comparisons. Nevertheless, the difference between the regions is highly significant by sign test ($P < .01$), with Michigan showing higher con-

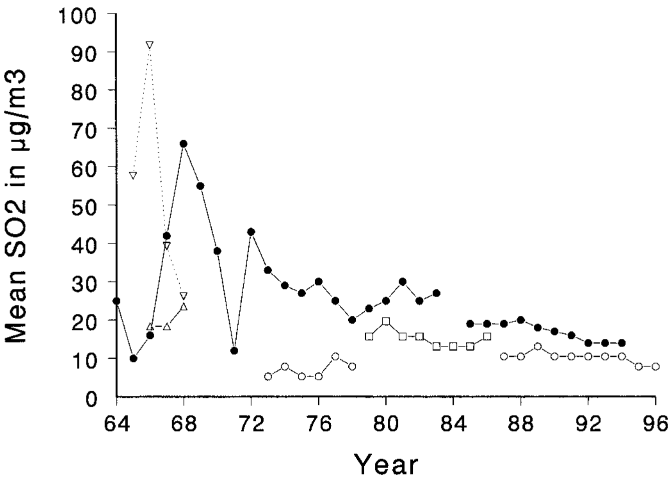

Figure 2. A comparison of atmospheric SO_2 concentrations in southeastern Michigan (solid circles) and western Virginia (open symbols). The Michigan records are from a single location near Detroit (Grant et al. 1996); the Virginia records are from three separate sites in the Roanoke area (open circles = Vinton, open squares = Salem, open upright and inverted triangles from two locations in Roanoke). Virginia records were provided by the Virginia Department of Environmental Quality.

centrations of SO_2 than Virginia in 23 of the 25 years measurements were taken in both places.

Other than statistically significant improvements in air quality, Grant et al. (1996) reported no visibly conspicuous environmental changes at the George Reserve. Here we note as a personal observation (from B.S.G.) that conspicuous changes have occurred in northeastern Pennsylvania following the collapse of the anthracite coal industry. Vast culm banks remain widespread throughout the coal mining region, but these once barren, black "mountains" of slack now are largely overgrown by stands of gray birch trees (*Betula populifolia*) that have expanded over the past 40 years. Kettlewell (1955) suggested that the light bark of birch trees affords suitable resting backgrounds for the typical phenotype of the peppered moth even in polluted woodlands. The recovery of typicals has also coincided with the rapid expansion of birch stands in parts of Britain following the Clean Air Acts (Grant and Howlett 1988). We do not suggest that the recovery of typicals is dependent upon birch trees, as typicals also thrive in mixed deciduous woodlands where birch species are not abundant (Grant et al. 1996); but birch trees, as do epiphytes in woodlands where they occur (Cook et al. 1999), contribute to the patterns and reflectance qualities of potential resting backgrounds used by adult moths.

Current levels of air pollution perhaps have fallen below biological significance regarding melanism in peppered moths, as present-day differences in SO_2 across regions no longer predict differences in the incidence of melanism. Both our Tennessee and West Virginia sites have current SO_2 levels exceeding our sites in Pennsylvania, Michigan, and Virginia, but unfortunately we have no historical data about melanism for Tennessee and West Virginia beyond our samples. In all cases the concentration of atmospheric SO_2 is below 30 $\mu g/m^3$, well below concentrations correlated with high fitness of melanic peppered moths in England (see Clarke et al. 1985). Current differences in melanism across regions likely reflect history more than contemporary environmental conditions.

The decline in melanism in the north over the past 40 years, and the near absence of melanism in the south might also be interpreted as evidence for thermal melanism, a phenomenon not uncommon in Lepidoptera (Majerus 1998), if our study were taken alone. However, there is direct evidence against thermal melanism in *B. betularia*; namely, the absence of latitudinal clines. This is clear from the geographic distribution maps showing the incidence of melanism in Britain (Kettlewell 1973) and Scandinavia (Douwes et al. 1976).

Both American and British peppered moth populations are converging on monomorphism for their respective typical forms, correlated with reduced levels of atmospheric pollution on both sides of the Atlantic. While a correlation alone does not establish a causal relationship, common correlations suggest a common cause.

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