# BISTON BETULARIA (GEOMETRIDAE), THE PEPPERED MOTH, IN WIRRAL, ENGLAND: AN EXPERIMENT IN ASSEMBLING

# CYRIL A. CLARKE

Department of Genetics and Microbiology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, England

# FRIEDA M. M. CLARKE

43 Caldy Road, West Kirby, Wirral L48 2HF, England

#### AND

### BRUCE GRANT

Department of Biology, The College of William and Mary, Williamsburg, Virginia 23185

ABSTRACT. The melanic form of the peppered moth, Biston betularia form "carbonaria," has continued to decline in frequency, comprising only 25.8% of a sample of 933 moths trapped on the Wirral peninsula near Liverpool, England, in 1991. The large sample was made possible, in part, by the use of two assembling traps and a mercury vapor trap. The assembling traps held either females of the North American subspecies, Biston betularia cognataria, or native British B. betularia females, thus allowing a comparison of the relative effectiveness of the two forms in attracting local males. Our results indicate that British B. betularia males do not discriminate between the mating pheromones released by the females of the two races.

Additional key words: "carbonaria," "cognataria," pheromone, industrial melanism.

Previous papers by Clarke et al. (1985, 1990) document the fall in frequency of the melanic form of the peppered moth, *Biston betularia* f. "carbonaria," at Caldy Common, West Kirby, on the Wirral peninsula near Liverpool, England, during the years 1959 through 1989. Figure 1 extends the census showing a slight "hiccup" in 1990 with "carbonaria" up from the previous year from 29.5% to 33.1%. The 1990 sample, however, was limited to only 154 moths, including 51 "carbonaria," 6 intermediates (=f. "insularia"), and 97 pale typicals. In 1991 "carbonaria" dropped precipitously to 25.8%, the lowest figure so far recorded.

The continued decline evidently reflects major habitat modification resulting from reduced industrial pollution accompanying the Clean Air Acts begun in the 1960's although it remains unclear what ecological factors are involved. The typical form of the moth, once widely thought to gain protection from predators by its resemblance to gray foliose lichens, is rapidly replacing "carbonaria" as the common form in the virtual absence of such lichens (Clarke et al. 1985, Grant & Howlett 1988). Clarke et al. (1985) also noted a gradual lightening of the trees in the absence of industrial soot, and Grant and Howlett (1988) have

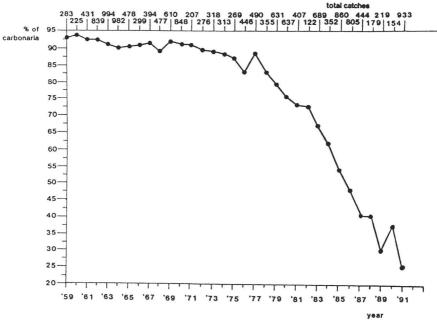


FIG. 1. The decline in the proportion of the melanic form of the peppered moth, *Biston betularia* f. "carbonaria," in West Kirby, Wirral, England. "Carbonaria" is expressed as a percentage of a total in which typicals and "insularia" are combined. The total number of *B. betularia* trapped in the 33 year period was 15,969 of which only 86 were female (0.54%).

further suggested that the comeback of birch trees (Betula pendula) following the Clean Air Acts may afford suitable hiding places for both the typicals and the melanics. Atmospheric sulfur dioxide concentrations at West Kirby have dropped markedly during this same period, and with minor fluctuations have remained low in recent years (see fig. 2 in Clarke et al. 1990). The mean winter  $SO_2$  levels ( $\mu$ g per cubic meter) were 20.17 in 1988, 25.25 in 1989, 31.89 in 1990 and the mean for January and February 1991 was 44.39. Whatever the cause, the decline in the frequency of the melanic variant of this species at this location continues.

A second component of our study involved the method of trapping. All 1990 moths were trapped by MV light (mercury vapor lamp) because we had no female *Biston betularia* available that year for use in an assembling trap (AT). The AT is a cage containing virgin females to which local males are attracted by airborne pheromones released by the captive females, i.e., the female odor serves to lure males to the trap. In 1991 we used both MV and AT methods, and we also had the

opportunity of altering the assembling techniques by using virgin female *Biston betularia cognataria* (Guenée) deriving from a large number of pupae brought by one of us (BG) from Virginia, USA.

Biston betularia cognataria is the North American equivalent of Biston betularia (L.). It has typical and melanic (f. "swettaria") forms, as well as intermediates grouped together as "insularia" (see West 1977). However, typical cognataria generally are darker in appearance than British typicals, and, in fact, have been described as resembling intermediate-grade British "insularia" (see Kettlewell 1973:plate 9.1). Kettlewell (1973) regarded cognataria and betularia as distinct species because of differences in color and in behavior in the early stages, there often being bivoltinism in the former but never in the latter. Rindge (1975), on the other hand, concluded that the evidence indicates that cognataria and betularia are members of the same biological species. They can be fully interbred and there is no disturbance of the sex ratio in the hybrids, and both male and female genitalia are identical or nearly so, as is the structure of the male antennae.

We used cognataria typicals from Virginia (about 20 at a time in the core of the trap and frequently replenished with fresh females as they emerged) to attract British B. betularia males throughout June 1991. Towards the end of this month the cognataria emergences were tailing off and the females old. Then our British B. betularia virgins (deriving from 20 pupae kindly supplied by Tony Liebert) began to eclose, and we used them in a different trap during July. Our original intention was to produce a quantitative bio-assay of relative pheromone effectiveness, but as we were unsuccessful in coordinating eclosions of the two kinds of females, we were unable to run both assembling traps simultaneously throughout both months. While the period of overlap when both cognataria and betularia females were available as lures in separate traps extended into July, we must emphasize that the number of individuals present in the cores, and their ages, unfortunately, were not controlled.

Nevertheless, the qualitative evidence that the *cognataria* pheromone is a powerful attractant to *betularia* males is compelling (Table 1). In June, when only *cognataria* females were releasing pheromone from the traps, 329 *betularia* males were drawn in as compared to only 109 males captured by the MV during that same period. During July, when both kinds of females were "calling" simultaneously from separate assembling traps, the numbers of males lured to the traps did not differ significantly by chi-square (144 to the *cognataria*-AT versus 171 to the *betularia*-AT,  $\chi^2 = 2.31$ , df = 1, 0.10 < P < 0.25). To our knowledge such data on comparative assembling have not been published previously, and, as we have no reason to conclude that the mating pheromones

TABLE 1. Biston betularia catches at West Kirby, Wirral, England, 1990 and 1991. Only the MV trap was used in 1990 as no virgin females were available. The 1991 combined catch figures also are subdivided to show numbers taken by each of the three traps used: a) MV trap; b) assembling trap using betularia females; c) assembling trap using cognataria females.

Year	Trap used	Total catch	Carbonaria	Insularia	Typical	% carbonaria
1990	MV only	154	51	6	97	33.1
1991	All traps combined	933	241	37	655	25.8
1991a	MV trap	289	78	15	196	26.9
1991b	betularia-AT	171	41	4	126	23.9
1991c	cognataria-AT	473	122	18	333	25.8

of *cognataria* and *betularia* are different, our findings are consistent with Rindge's (1975) assessment that *cognataria* and *betularia* are conspecific.

It is true that pheromones occasionally are not entirely species specific, because Clarke (1979) showed that Orgyia thyellina Butler (Lymantriidae) females from Japan regularly assembled Orgyia antiqua (L.) males in the Wirral, though this is probably an exception to the general rule. The point, however, must be made that Priesner (1975) thought the pheromones of all Orgyia species were similar, but the findings were made by antennogram techniques which are not particularly sensitive. If the pheromones in the genus Orgyia were alike there would be mating chaos where species fly together, and we know from observation that O. recens Hübner and O. antiqua do not attract each other (Greenberg et al. 1982).

A final point in the 1991 series relates to the placement of the three traps. Jones, Majerus, and Timmins (unpublished) have produced evidence that in five polymorphic moth species in which melanism is thought to be of ancient origin, great sampling differences in morph frequencies over very short distances occur depending on the local environments in which traps are placed. They suggest that such habitat selection is not likely to be present where the melanism is of recent origin, as in *Biston betularia*, and our 1991 data support this view. Specifically, our MV trap was completely in the open, the *cognataria*-AT under a very thick, old oak tree, and the *betularia*-AT in a relatively exposed position near a small birch tree. Yet, all three morph frequencies were proportionately represented in all three trapping locations. In fact, the three data sets are remarkably homogeneous by G-test (G = 3.28, df = 4, 0.5 < P < 0.75).

In summary, the 1991 sample of 933 Biston betularia at West Kirby shows a return to the lowering of the proportion of f. "carbonaria," and we report that much of the assembling data resulted from using the American subspecies, B. b. cognataria.

# ACKNOWLEDGMENTS

We are grateful to Tony Liebert for providing B. betularia pupae; Sally Thompson for technical assistance throughout the study; R. J. Agass, Director of Housing and Environmental Protection, Metropolitan Borough of Wirral, for the  $SO_2$  levels; M. E. N. Majerus for sending us a draft of his submitted manuscript; and the Nuffield Foundation for support.

# LITERATURE CITED

- CLARKE, C. A. 1979. Some observations on *Orgyia thyellina* Butler and *Orgyia antiqua* (L.). Entomol. Rec. 91:315–316.
- CLARKE, C. A., G. S. MANI & G. WYNNE. 1985. Evolution in reverse: Clean air and the peppered moth. Biol. J. Linn. Soc. 26:189–199.
- CLARKE, C. A., F. M. M. CLARKE & H. C. DAWKINS. 1990. Biston betularia (the peppered moth) in West Kirby, Wirral, 1959–1989: Updating the decline in f. carbonaria. Biol. J. Linn. Soc. 39:323–326.
- Grant, B. & R. J. Howlett. 1988. Background selection by the peppered moth (Biston betularia Linn.): Individual differences. Biol. J. Linn. Soc. 33:217-232.
- GREENBERG, S., A. H. WRIGHT & C. A. CLARKE. 1982. Contrasting results in assembling experiments using *Orgyia thyellina* Butler, *O. recens* Hübner and *O. antiqua* (L.). Entomol. Rec. 94:25–27.
- KETTLEWELL, B. 1973. The evolution of melanism. Clarendon Press, Oxford. 423 pp. PRIESNER, E. 1975. Electroantennogram responses to female sex pheromones in five genera of Lymantriidae (Lepidoptera). Z. Naturforsch. 30:676–679.
- RINDGE, F. H. 1975. A revision of the New World Bistonini (Lepidoptera: Geometridae). Bull. Am. Mus. Nat. Hist. 156:69–155.
- WEST, D. A. 1977. Melanism in *Biston* (Lepidoptera: Geometridae) in the rural Central Appalachians. Heredity 39:75–81.

Received for publication 17 March 1992; revised and accepted 31 August 1992.