Selective bird predation on the peppered moth: the last experiment of Michael Majerus

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Colour variation in the peppered moth Biston betularia was long accepted to be under strong natural selection. Melanics were believed to be fitter than pale morphs because of lower predation at daytime resting sites on dark, sooty bark. Melanics became common during the industrial revolution, but since 1970 there has been a rapid reversal, assumed to be caused by predators selecting against melanics resting on today’s less sooty bark. Recently, these classical explanations of melanism were attacked, and there has been general scepticism about birds as selective agents. Experiments were often placed on tree trunks, which were argued to be abnormal resting sites; pinned carcasses seemed particularly unnatural. In experiments, moths were often released at greatly inflated densities, which were argued to be abnormal resting sites; pinned carcasses seemed particularly unnatural. Moths were often released at greatly inflated densities, potentially increasing predation. Reared insects from geographically distant sources were often used to supplement wild individuals, and may not have behaved as naturally in recapture experiments as wild moths. By the 1990s, considerable scepticism became evident [11–14]. Factors other than bird predation (e.g. migration, physiological differences among genotypes) were argued to play a substantial role in the evolution of melanism in Biston [5,15–17]. Caveats about the predation experiments discussed in Majerus’s book [5], critiques by other biologists, as well as points made particularly forcefully in a review of the Majerus book [18], were soon exploited by non-scientists to promote an anti-evolution agenda and to denigrate the predation explanation [10]. Kettlewell’s original mark–recapture experiments were later argued to be fraudulent [19] (quite groundlessly: see [3,10,20]). Judith Hooper, author of this claim, also suggested that bats rather than birds might be the agents of selection [19]. Soon, both the public in general and even evolutionary biologists began to doubt the bird predation story (electronic supplementary material, S1 [18]).

Majerus therefore decided to make key new field observations, and he also designed and carried out a massive new predation experiment, the largest predation experiment ever performed (4864 released moths) to answer his own and other criticisms of earlier work [5,10,21]. In addition, to address the possible effect of bat predation, Majerus released live moths in night-time experiments in multiple locations: he found no significant differences in predation of 419 melanic and typical moths eaten by three species of pipistrelle bats [22].

These results were all presented by Majerus in a keynote address at the ESEB Congress, Uppsala, in
August 2007. Unfortunately, Majerus died after a short illness in 2009 before publishing the rest site observations and predation results. However, the information from the Uppsala talk, which forms the basis for the current analysis, was made freely available by Majerus on the Internet soon thereafter as a set of projected slides. We have formed the current collaboration in order to analyse these results and disseminate them in print for the first time, as well as to clarify the importance of these key results for our understanding of natural selection in the wild.

### 2. MATERIAL AND METHODS

Experimental and observational work presented here was carried out by Michael Majerus in a 1 ha rural garden, at Springfield, near Coton, Cambridgeshire, UK. Full methods were published in a little-known chapter [10]; relevant extracts are provided here (electronic supplementary material, S2). Original source files of his presentation in 2007, which contain the results analysed here, are provided at http://dx.doi.org/10.5061/dryad.962262h9. In cases where numerical data were not supplied in these files, we have expressed the results in terms similar to those used by Majerus in the 2007 documents.

(a) **Natural resting sites**

While climbing trees in the experimental site in order to set up sleeves for the predation experiment (see below), Majerus systematically scrutinized trunks, branches and twigs of a limited set of trees and recorded natural resting positions of all wild moths he found. The 135 observations he obtained here add considerably to the less-extensive resting site data previously published [10,12].

(b) **Predation experiment**

The purposes of the new experiment by Majerus were to estimate the relative survival of local melanic and non-melanic moths at low, naturalistic densities. Moths (melanic *carbonaria* and non-melanic *typica*; no *musseliana* morphs were used) were therefore released on substrates that Majerus himself had shown were normal for the species. Morphs were released at frequencies close to those estimated from captures in the previous year at Madingley, near Cambridge. (These Madingley frequencies have been interpolated by us from a graph provided by Majerus in his 2007 presentation; see table 2, column 2).

A total of 4864 peppered moths were released during the natural emergence seasons over 6 years. Each night one moth was released into each of the 12 netting ‘sleeves’ surrounding a branch selected randomly from among the 103 (reduced to 97 by 2007) branches used in the study. Sleeves and any moths resting on them were removed before dawn, and positions of moths remaining undisturbed on bark were noted. Release density averaged less than 10 moths ha\(^{-1}\) night\(^{-1}\).

Moths absent from resting positions 4 h after sunrise were presumed eaten by predators as they rarely fly away during daylight unless greatly disturbed. Of those that disappeared, approximately 26 per cent were seen being eaten by birds via binocular observations [10]. For further details, see electronic supplementary material, S2 and the study of Majerus [10].

(c) **Statistical analysis of predation experiment**

Data from the predation experiment (table 2, columns 4–6) were provided as a three-way contingency table. The numbers of *carbonaria* and *typica* released yearly were fixed by the experimental design. Such non-standard contingency tables are readily handled by log-linear models [23]. Maximum-likelihood values of parameters of non-selective and selective predation rates were estimated given the assumptions of homogeneity or heterogeneity of effects among years, and likelihood-ratio tests were performed to test models of different hierarchical complexity [24,25]. While simple contingency table analysis is probably more familiar, non-selective predation was heterogeneous among years, and a more complex analysis that takes account of the heterogeneity was required (see electronic supplementary material, S3).

### 3. RESULTS

(a) **Natural resting sites**

Majerus had already shown that some moths did normally rest on tree trunks during the day [10].

<table>
<thead>
<tr>
<th></th>
<th>trunks</th>
<th>branches</th>
<th>twigs</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>males</td>
<td>28</td>
<td>40</td>
<td>11</td>
<td>79</td>
</tr>
<tr>
<td>females</td>
<td>20</td>
<td>30</td>
<td>6</td>
<td>56</td>
</tr>
<tr>
<td>totals</td>
<td>48</td>
<td>70</td>
<td>17</td>
<td>135</td>
</tr>
</tbody>
</table>

His recent extensive observations on resting sites obtained while climbing the trees in his garden to set up the predation experiment appear to have been lost, but he summarized the work in his 2007 Uppsala presentation (http://dx.doi.org/10.5061/dryad.962262h9). An annotated version of this summary is presented here. Majerus reported the following major features (table 1):

- The majority (52%) of moths rest on lateral branches.
  - Of the moths on lateral branches, the majority (89%) rest on the lower half of the branch.
  - A significant proportion of moths (35%) rest on tree trunks.
  - Of those that rest on trunks, the majority (87%) rest on the north, rather than the south half.
  - A minority of moths (13%) rest under or among twigs.
  - There were no significant differences in the resting sites of males and females ($\chi^2 \approx -2\ln L = 0.33, 2 \text{ d.f.}$).
  - There were no significant differences in the resting sites used by *typica* (non-melanic), *carbonaria* (full melanic) or *insularia* (intermediate melanic) forms (numerical data not available).

The findings about orientation of resting position suggest avoidance of exposure to sun, while also verifying that a reasonably high fraction of moths do rest on the trunks of trees, as well as in the canopy.

(b) **Predation experiment**

In Majerus’s new predation experiment at an unpolluted site, significantly more released melanics than non-melanics disappeared, or were seen to be eaten by nine bird species (figure 1 and table 2). This lower fitness of melanics is expected if the observed decline in melanism (table 2) is explained by visual predation.

The full statistical analysis (electronic supplementary material, S3) demonstrates:

- strong evidence of heterogeneity in the non-selective fraction eaten of both morphs across years ($p < 0.001$), but
- no significant variation in the fraction selectively eaten across years, and
The lifespan of wild moths is several days, so the approximately 9 per cent reduction in daily survival of melanics is sufficient in magnitude and direction to explain their long-term local decline; the decline of melanics is sufficient in magnitude and direction (table 2; [3]). Majerus was able to see predation events from his window, involving nine species of local insectivorous birds (table 2). Clearly melanics disappeared faster than non-melanics in this experiment, and Majerus was able to confirm by direct observation that about one-quarter of the disappearances were owing to bird predation [10].

Factors other than predation have often been argued to play a substantial role in the rise and subsequent post-industrial fall of melanism in Biston [5,15–17]. Nonetheless, with this new evidence added to the existing data, it is virtually impossible to escape the previously accepted conclusion that visual predation by birds is the major cause of rapid changes in frequency of melanic peppered moths [3,5]. These new data answer criticisms of earlier work and validate the methodology employed in many previous predation experiments that used tree trunks as resting sites [3]. The new data, coupled with the weight of previously existing data convincingly show that ‘industrial melanism in the peppered moth is still one of the clearest and most easily understood examples of Darwinian evolution in action’ [21].

We thank Ziheng Yang, Sami Merilaita, Anders Forsman, Remy Ware, Chris Jiggins, Frank Jiggins, Blanca Huertas, the family of Michael Majerus and two anonymous reviewers for help and suggestions. We are grateful for permission from Michael Majerus to make the data available in the current format. Deposited data: http://dx.doi.org/10.5061/dryad.962262b9

### 4. DISCUSSION

The lifespan of wild moths is several days, so the approximately 9 per cent reduction in daily survival of melanics is sufficient in magnitude and direction to explain their long-term local decline; the decline rate suggests a selection pressure against melanics of \( s \approx 0.1–0.2 \) per generation (table 2; [3]). Majerus was able to see predation events from his window, involving nine species of local insectivorous birds (table 2). Clearly melanics disappeared faster than non-melanics in this experiment, and Majerus was able to confirm by direct observation that about one-quarter of the disappearances were owing to bird predation [10].

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**Table 2. Survival of moths in the predation experiment in different years.** Column 2: Frequency of wild melanics (carbonaria) obtained in light trap samples at Madingley, near Cambridge. Columns 3–6: Typical and melanic (carbonaria) individuals made available and eaten at the experimental site in suburban Cambridge, UK. Expected values under null (\( s \approx s \)) and best-fit (heterogeneous overall survival and homogeneous predator selection among years, \( s \approx s \)) models are shown, respectively, in parentheses. Nine bird species were observed eating the moths: English robins (Erithacus rubecula), hedge sparrows (Prunella modularis), great tits (Parus major), blue tits (Cyanistes caeruleus), European blackbirds (Turdus merula), starlings (Sturnus vulgaris), Eurasian wrens (Troglodytes troglodytes), magpies (Pica pica) and a lesser-spotted woodpecker (Dendrocopos minor).

**Figure 1. Survival of moths (\( s \approx s \)) over the course of the predation experiment.** Unfilled diamonds with dashed lines, non-melanic; filled squares with solid lines, melanic.

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