Selective Bird Predation on the Peppered Moth

Electronic supplementary materials ESM2

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Details of Majerus' experimental methods, including reasons given for doing the experiment, with notes added by the authors

1. Introduction

Majerus felt that previous predation experiments on the peppered moth were insufficiently documented, and he therefore took the unusual step to publish rather full details of his methodology and its reasoning while the experiment was under way. In addition, he wished to publish the methods in advance of the results in order to open up the methods to criticism of others. These methods were eventually published in a book chapter [1]. The writing of this chapter finished only two years into the experiment (2003), so few results were available, and none were published at this time. His sudden illness and death in 2009 precluded publication of any results.

The experiments and observations reported in the main paper were designed by Majerus in response to a number of criticisms of previous experiments, including those that he himself had raised [2]. Perhaps the most iconic of these experiments were also the first [3,4]. Major criticisms were as follows (transcribed from ref. [1], pp. 379-380):

> **1**. The densities of moths in Kettlewell's predation and mark-release-recapture experiments were too great.
> **2**. Kettlewell released moths on to tree trunks. The evidence that exists suggests that although some peppered moths naturally rest in exposed positions on tree trunks, this is not their preferred resting site.
> **3**. In his mark–release–recapture experiments, Kettlewell released moths during the day. Peppered moths prompted to fly during the day will settle on the first substrate that they encounter, and generally remain still thereafter. Thus, moths released during daylight will not select the same sites as those that settle at the end of night flight. It is improbable that the degree of crypsis secured by Kettlewell's released moths would have been as high as that of moths in the wild.

> **4**. Kettlewell used mixtures of wild-caught and laboratory-bred specimens, which may have behaved differently.
> **5**. The moths that Kettlewell released in Birmingham and Dorset may not have originated in the same locations, and so may have had local behavioural adaptations.
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> In addition, many later workers glued dead moths on to trees in 'life-like' positions, selecting sites that maximized their crypsis. I have tried to do this by very carefully gluing moths on to birch tree trunks, and releasing a similar number of live moths on to the trunks soon after dawn. A class of students then assessed the degree of crypsis of the moths by walking towards the trunks and saying when they could see any moth. For all forms, the live moths were more cryptic than the glued moths.
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> With one exception, one or more of the studies subsequent to Kettlewell's avoids each of these sources of artificiality, and shows that they do not affect the differential bird predation hypothesis. The only criticism that can be aimed at all the predation studies conducted to date is that the moths available for predation did not take up their own resting positions during the pre-dawn flight that characterizes this species. This criticism should be addressed in future predation experiments.

2. Observations of natural resting sites

Majerus described his methods for observation of resting positions as follows (ref. [1], pp. 391-392):

> The slowest accumulating dataset that I have is of the resting positions of peppered moths I have found in the wild since 1964. This dataset, first published in 1987 [5], has continued to build. The set up to 2001 is given in Table 12.4a, and consists of just 59 moths, a rate of 1.55 moths located per year. While constructing or removing release sleeves in the trees, I have found, by eye, a considerable number of moths, 27 of which have been peppered moths (Table 12.4b). The rate of find has thus risen to 13.5 moths per year.

In Table 12.4b of ref. [1], it is clear that these 27 moths were found while investigating and climbing in the trees to affix the netting arenas he needed for his experiments. They are described in the Table as:

> moths found while climbing trees or working in the canopy to construct or remove release sleeves, 2002-2003 (Springfield, Cambridge).

And from his 2007 slide presentation in Uppsala, describing the main data we present in Table 1:

> During the main predation experiment, I have had occasion to spend time carefully scrutinizing the trunks, branches and twigs of a limited set of trees at the experimental site. During this time I have found 135 peppered moths, resting in what I have no reason to presume are not their freely chosen natural resting sites.

We therefore infer that of the 135 moths in Table 1 in the main paper, at least 27 of them were formerly mentioned in Table 12.4b of ref. [1].

In the presentation of 2007, there were two trivial inaccuracies in percentages of moths Majerus reported on different substrates, given the numerical data also presented (Table 1). In our presentation of results (in the main paper), we have corrected these errors and rounded to the nearest 1%.

3. Predation experiment

Majerus described the methods for the main predation experiment as follows (transcribed from ref. [1], pp. 389-391):

> **A new predation experiment**
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> To determine whether changes in *carbonaria* frequency can be accounted for by differential bird predation requires a predation experiment that avoids the suggested flaws in those carried out by Kettlewell and others. Such an experiment was designed in 2001. The design took account of the criticisms aimed at previous experiments, plausibility of procedure and methods of statistical analysis. Initial testing of release procedures, the trees that peppered moths rest upon in the area, the visibility of subjects during experiments and levels of predation, and [sic] was undertaken in 2001, in Madingley Wood, to assess feasibility.
>
> The experimental design is as follows:
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> **Location**: A 1-ha garden plot. The garden is surrounded by a mature hawthorn / cherry / dog-rose / sallow / ash / oak / elder / sloe / leylandii / privet boundary hedge. Approximately half of the plot comprises old orchard (apple, pear, plum) and mature deciduous trees (oak, birch, willow, goat sallow). Agricultural land, mature deciduous hedges and small areas of deciduous woodland surround the garden.

Release positions: 103 branches were identified and numbered [i]. Branches vary in their angle to the horizontal from 79° to -37°, sourcing from the trunk. All release positions were on native trees. The height above ground of the positions varies from 1.8 to 26 m. Lower positions are reached by ladders. Climbing aids have been attached to trees (or adjacent trees) to allow access to higher positions.

The method of release uses 0.5–1-m-long black netting sleeves placed around branches. Wire rings are set around the branches to produce a netting cylinder with the netting proud of the branch by approximately 20 cm. The ends of the cylinders are tapered and elasticated so that they fit tightly around the branch. Each evening, 12 such cylinders are constructed. The positions for the cylinders are chosen by random numbers from the 103 release sites [1]. One peppered moth, either *betularia* [ii] or *carbonaria*, is released into each cylinder within the hour prior to sunset. The cylinders are carefully removed the following morning, during the 40 minutes prior to sunrise. The positions of the moths are noted. Moths disturbed during this procedure, or not resting on the branches, are removed. This removal, on average, comprises about one-third of the moths, leaving a release density of about 8 moths/ha. Period of release is during the period that the peppered moth is on the wing in the area (determined as the average of the earliest and latest record in fight traps over the previous 20 years), i.e. 11 May until 19 August.

The moths are all of Cambridge origin (within 5 km of the release site). Only *carbonaria* and *betularia* [2] forms are used. Moths are used at the relative frequencies that they occurred in the area in the previous summer (determined by running a set of four light traps and four pheromone traps, at Madingley Wood, 1.9 km from the release site). The moths used are of four types [iii], used in approximately equal numbers:

1. Moth-trap-caught males.
2. Pheromone-trap-caught males.
3. Bred males that had eclosed on the day of use.
4. Bred females that had eclosed on the day prior to use and had mated the previous night in cages hung outside at another location (Genetics Field Station).

The main dispersal flight of females takes place on the second night after eclosion, having mated the first night (Liebert and Brakefield, 1987)[iv].

The genotypes of bred males and females used are known. The genotypes of the moths released are in the approximate proportions that the genotypes occur in the area.

Recording of predation: 58 of the release positions are visible from the house at the centre of the garden. As moths do not always rest on a part of the branch visible from the house, the number of released moths that can be kept under direct observation varied, but over the test period (2001) it was 29%, and over the first two years of the experiment (2002-2003) it has been 26% of those released. Observation is aided by binoculars or a telescope. Any moths taken by birds are recorded, as is the species of bird. Any moths seen to change position are also recorded.

Observations commence immediately after the last cylinder is removed, and continue for between 3 and 4 h. Approximately 4 h after sunrise, each release site is visited, and a record made of whether the moth is present or absent [v]. All remaining moths are removed to prevent an accumulated increase in the density of moths in the garden.

Analysis of the results will consider the relative rates of predation of the two forms. The question of whether these rates can account for the declining frequencies of *carbonaria* in the Cambridge area will be addressed. In addition, data from the four types of moth used will be compared, as will be data from observed and unobserved disappearances [3].

The design of this experiment tries to circumvent as many of the criticisms aimed at Kettlewell's predation experiments as possible. Thus the design uses moths released at low density, at natural frequencies, on to natural resting sites in such a way that they choose their own resting position, albeit in a restricted arena. The design also only uses moths originating from the area of the release site, and should allow determination of differences between both male and female moths and light-trap-caught, pheromone-trap-caught and bred moths [3].

Due to the low frequency of *carbonaria,* and the current rate of decline of *carbonaria,* which gives a fitness disadvantage for *carbonaria* of approximately 0.15, the experiment will run for 5 years [vi].

Airing the experimental design here has the aim of avoiding future accusations, of the type aimed at Kettlewell, of altering the design as the work progresses [9,10].

4. Results presented during the keynote lecture of 2007, Uppsala, Sweden

The only results we have available were presented by Majerus in a keynote address at the ESEB Congress, Uppsala, in August 2007 (as already noted in the main article).

In his presentation, Majerus appears to have incorrectly estimated selection, given the numerical data he presented in the same lecture (table 1). In the main paper, we have therefore corrected this error using our fuller statistical analysis.

In addition, Majerus noted a weak correlation between the decline of moths from year to year and the selection pressure estimated from the year before. While there is a weakly positive value of the correlation coefficient (even after correcting the measure of selection), there is no significant variation in selection from year to year (see Results), and the correlation estimate itself has only 4 degrees of freedom and is very far from significant. Accordingly, we do not report on this insignificant result in the main text.

Footnotes

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[i] In his 2007 presentation, Majerus records that the experiment was run with these 2005 methods, except:

> Only changes during experiment: i) the number of release sites has dropped to 97 due to storm damage, and ii) the experiment ran for six years, rather than five, due to low frequency of *carbonaria* in 2003.

We also find in the 2007 document that he used a release of "<10 per hectare per night" rather than "about 8 moths/ha" as stated in the 2005 methods.

[ii] Majerus uses '*betularia*' here to mean non-melanic moths, which more usually are termed '*typica*.'

[iii] Majerus evidently intended to record which individual predation event was on which morph and sex of moth, and from which individual location [1,6]. He did this in part to answer his own criticism of Kettlewell's experiments; Kettlewell had used a mixture of wild caught moths, supplemented with reared individuals, for his release experiments. Whether rearing affected recapture probability was the subject of a later mark-recapture experiment: no evidence was found that reared and natural moths differed in recapture rates [7].

However, Majerus' records on this issue appear to have been lost, and he did not present any of these details in 2007. In the current experiment, as the resting sites were randomized, and as we doubt that rearing vs. wild capture would have much effect on the resting position adopted by moths within the enclosed space of the sleeves at each position, we do not believe that knowledge of the types of moths would have much effect on the results. (In contrast, it does seem rather more likely that rearing might affect recapture rates in freely-flying moths, as in Kettlewell's and Bishop's experiments). In view of the wide confidence intervals of the estimates, we believe that, even if we had the records, there would be little power to test among rearing methods or factors other than those tested here.

[iv] Actually, Liebert & Brakefield [8] report that females use the first night to disperse and mate on their second night.

[v] After a *Biston* moth settles at dawn, or is placed on a tree during daytime, it very rarely flies off, in our experience. Thus most of the disappearances recorded are due to being eaten by predators.

[vi] As Majerus predicted, after five years it became very difficult to release reasonable sample sizes of the *carbonaria* morph, since he aimed to release moths at the frequency estimated from the previous year's captures in the Cambridge area. In total, six years' data were recorded rather than the five suggested in ref. [1] (see note 1). By 2007, the last year of his experiment, only 14 *carbonaria* moths were released under this restriction, giving very little power to estimate selection.

References

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