**PP1** The Peppered Moth: The Proof of Darwinian Evolution

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Thankyou XX

I am delighted to be here at my first ESEB conference. Today I want to talk to you about one of the best known, and, over the last decade, most controversial examples of Evolution in Action, that of industrial melanism in the Peppered moth, *Biston betularia*.

The case reached a prominence as one of the most cited examples of Darwinian evolution in action because of the visual impact of the case, the ease with which it can be understood by non-specialists, and because empirical evidence was collected, in the 1950s, to show the selective agent responsible for the changes that had been observed.

I am sure that most of you know the peppered moth story, but just as a resume, in brief the story was this:

**PP2** The non-melanic, or *typica* peppered moth is white, liberally speckled with black. **(*Typica*)**

**PP3** In 1848, a black form, f. *carbonaria* was recorded in Manchester. **(Fact + carbonaria)**

**PP4** By 1895, 98% of the Mancunian peppered moths were black. The *carbonaria* form spread to many other parts of the UK, reaching high frequencies in industrial centre and regions downwind. In 1896, the great Victorian lepidopterist, J.W. Tutt hypothesized that the increase in *carbonaria* was the result of differential bird predation favouring *carbonaria* in polluted regions, but not in unpolluted regions. Nothing much happened for the next half century, except that Haldane showed that *carbonaria* would have to have been one and a half times as fit as the typical form, to explain its observed increase in frequency in Manchester.

In the 1950s, Bernard Kettlewell obtained data from direct predation experiments, and mark-release-recapture experiments, in two woodlands, one polluted, the other relatively unpolluted, that supported Tutt's hypothesis.

**PP5** It was the reciprocal nature of Kettlewell's results in the two woodlands, allied to extensive survey work showing a strong correlation between *carbonaria* frequency and industrial pollutants that made

the case so persuasive. Over the next 40 years, various researchers worked with peppered moths to tease apart the fine detail of the case, but none of the new findings seriously undermined Tutt's hypothesis or Kettlewell's evidence in support of it.

**Need NEW YORK TIMES**

**PP6** The zenith for the case came in 1996, when, reporting work from both sides of the Atlantic that showed similar changes in melanic frequencies were correlated to pollution levels (Grant et al., 1996), *The New York Times* featured the peppered moth on the front page of its science section.

However, since Kettlewell's experiments, the black peppered moth has suffered two types of decline. First, following anti-pollution legislation in the 1950s and thereafter, *carbonaria* began to decrease in frequency, as would be expected from Tutt's theory.
Second, the reputation of the case as an example of Darwinian evolution in action has been severely tarnished.

**PP7** Today, I want to briefly explain the reason for the case's decline in reputation.
I will then detail why I chose to undertake a piece of experimental work that has taken me 7 years, before presenting you with a series of observations on the natural resting sites of the peppered moth, and the results of two field experiments.
Finally, at the end, I may make a few mild concluding remarks.

**PP8** The decline in the peppered moth's reputation may be sourced to a book that I wrote in 1998 **(show book)**, or, more correctly **PP9** a review of it by Jerry Coyne, in *Nature*, in which Coyne concluded *'…. For the time being we must discard Biston as a well-understood example of natural selection in action…*'.
I, and others, have dealt with this review previously, showing that the review had little to do with what was said in the book. As Donald Frack put it *"There is essentially no resemblance between Majerus' book and Coyne's review of it. If I hadn't known differently, I would have thought that the review was of some other book."*

**PP10** But the damage had been done. Coyne's review, and an article, titled *"Scientists pick holes in Darwin moth theory"* by Robert Matthews in the Sunday Telegraph (March, 1999), began to appear on creationist and anti-evolution web-sites.

**PP11** The creationists began to smell blood, and a series of articles appeared with titles such as: **(*Second thoughts about the peppered moth*, Darwinism in a flutter, *The moth that failed*, Staple**

**of evolutionary thinking may not be a textbook case, *Moth-eaten statistics*, The Piltdown moth, *Goodbye, peppered moths; a classic evolutionary story comes unstuck*).**

I should point out that it was about this time, the year 2000, that I first began to formulate the design of the predation experiment that I will tell you about later. But before we come to that, I must mention one other publication that raised the ante.

**PP12** In 2002, Judith Hooper, an American journalist and writer, published a book called *Of Moths and Men: Intrigue, Tragedy and the Peppered Moth*, which is, according to the front cover, a riotous story of ambition and deceit.
This appalling book is essentially an attack on the peppered moth case, those who have worked on the evolution of industrial melanism, lepidopterists in general, and Kettlewell and Professor E.B. Ford in particular.

**Need Hooper Book**
**PP13** To cite the front fly-sheet, "*Of Moths and Men* is … a fascinating psychological dissection of the ambitious scientists who will ignore the truth for the sake of fame and recognition".

**PP14** I do not want to dwell on Hooper's book for long. Various reputable scientists, from both sides of the Atlantic: Bruce Grant, Bryan Clark, Lawrence Cook, James Mallett, Paul Brakefield, David Rudge, myself, and even Jerry Coyne, who I think might have been feeling a bit guilty about his review of my book, have discussed the many flaws in Hooper's book. Coyne, for example, in a review in Nature criticizes her *'flimsy conspiracy theory'*, her theme of *'ambitious scientists who will ignore the truth for the sake of fame and recognition'*, by which *'she unfairly smears a brilliant naturalist'*

**PP15** Coyne concludes: "'*This issue matters, at least in the United States, because creationists have promoted the problems with* Biston *as a refutation of evolution itself. Even my own brief critique of the story has become grist for the creationists' mill. By peddling innuendo and failing to distinguish clearly the undeniable* ***fact*** *of selection from the contested* ***agent*** *of selection, Hooper has done the scientific community a disservice*.'

**Refer to Hooper Book**
**PP16** So I will give just a single example of the standard of Hooper's book. If you go to the first page of Chapter 1, the first sentence is, "*To begin at the beginning, the Lepidoptera are divided into two orders: the butterflies (Rhopalocera) and the moths (Heterocera)*. Sadly, the scientific accuracy of the book rarely rises from this inauspicious start.

**PP17** I leave it to David Rudge to comment of the veracity or otherwise of what Bruce Grant calls Hooper's "relentless suspicion of fraud" aimed at Kettlewell. Rudge (2005) who examines Hooper's evidence that Kettlewell committed scientific fraud concludes *"that Hooper does not provide one shred of evidence to support this serious allegation".*

**PP18** In 2000, while the peppered moth was under initial attack from anti-evolution lobbyists, I conceived two parts of the work that I am going to describe to:
1 Fill up a major gap in our knowledge of the natural history of the peppered moth: that is, where peppered moths rest in the day, and
2 Check whether various valid criticisms of Kettlewell's experimental protocols could have altered the qualitative validity of his conclusions, by conducting a new field predation experiment.
Hooper's book caused me to include an additional experiment, involving peppered moths and bats.

**PP19** The question that I wished to answer with the main predation experiment was: **Is differential bird predation sufficient to explain any changes in the frequencies of the *typica* and *carbonaria* forms observed over a period of years.** Given previous observations in the Cambridge area and other parts of Britain, I knew that the frequency of *carbonaria* had been declining, and I had no reason to suppose that the decline would not continue.
N.B. It was not possible to replicate Kettlewell's reciprocal design because in no part of Britain is *carbonaria* frequency increasing.

**PP20** The main experiment, was designed to take account of all the flaws that had been aimed at Kettlewell's work, that:

i) The densities of moths were too great, and he used too few release sites
ii) Moths were released onto tree trunks, when Kettlewell knew that peppered moths usually rest under lateral branches
iii) Moths were released during the day, and so might not have selected sites that would maximize their crypsis
iv) Kettlewell used mixtures of wild caught and lab bred moths, which might behave differently
v) Kettlewell used translocated moths that might have had different behaviours as a result of local adaptation.

**PP21** My experimental design, which was piloted in 2001, and has already been published (Majerus, 2005) allowed me to:

i) Do the experiments in the wild, at low frequency (<10 per hectare per night), and collect back any moths left at each predation run.

ii) Release moths in their natural resting positions (initially 103 release sites in a 1 hectare experimental site)

iii) Release moths at dusk, into restricted arenas at their natural resting sites, so that they could take up resting positions at the end of their night flight. Arenas are removed in the forty minutes before sunrise.

iv) Use and compare moths that were moth-trap caught males, pheromone-trap caught males, lab-bred males or lab-bred females.

v) Only use moths from Cambridge, within 5km of the experimental site.

**PP22** In addition, I:

vi) Released moths at the frequencies that they occurred in the previous year at a site 1.9km from the experimental site.

vii) Ran the experiment during the months that the moth is naturally on the wing.

viii) Predation was scored by direct observation or absence after 4 hours.

This experimental procedure has been adhered to, with the only changes being that 6 of the release sites were lost due to storm damage, and the experiment, which initially was expected to last for 5 years, has taken 6 because of the low frequency of *carbonaria* in 2003, which meant that the number of *carbonaria* being exposed to predation was lower than expected.

**PP23** This is the experimental site.

**PP24** The additional experiment that has been done addresses the question of bat predation. This arose directly from Hopper's book, for it reveals Hooper's lack of understanding of Darwinian selection. Hooper (2002, p. 270) raises the question of bats as predators of peppered moths. She states that "Kettlewell himself admitted that they {bats} probably accounted for 90% of the predation of adult moths."

By e-mail in 2000, she pointed out to me that Kettlewell had "said that this didn't matter because it wasn't selective—ergo, even if only 10% of the predation was by birds hunting by sight, that 10% is what makes the difference and drives evolution". Hooper thought that there were flaws in this argument and asked me about this.

By phone I said I agreed with Kettlewell and explained why (Hooper, 2002, p. 270). But not understanding how selection operates, Hooper didn't get it, and concludes, 'Can we really be sure that bat predation is not selective….?.

**PP25** For bats hunting by sonar, it is hard to see how they could be responsible for the changes in *carbonaria* frequency correlated to pollution levels, without some fairly unrealistic logical gymnastics and assumptions. But, I decided to do an experiment anyway: to test whether bats do prey on *typica* and *carbonaria* differentially.

The design was simply to release equal numbers of the forms near moth-traps where pipistrelle bats were feeding and watch which moths were eaten.

**PP26 Bat data** From the data shown here, it is obvious that there are no significant differences in the predation of the two forms. Across the four runs, 208 *carbonaria* and 211 *typica* were taken.

**PP27** So pipistrelle bats do not show differential selection of *typica* compared to *carbonaria* or vice-versa.

**PP 28** 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.

**PP29** The position of each moth was scored for resting site (trunk, branch, twig); height above ground; on trunks, north or south half; on branches, top or bottom half. Sex and form of each moth was also recorded.

**PP30** The results are here.

**PP31** In summary, these results show that:

i) The majority (50.4%) of moths rest on lateral branches.
ii) Of the moths on lateral branches, the majority (89%) rest on the lower half of the branch.
iii) A significant proportion of moths (37%) do rest on tree trunks (so Kettlewell wasn't so wrong in releasing his moths onto tree trunks).
iv) Of those that rest on trunks, the majority (86.8%) rest on the north, rather than the south half.
v) A minority of moths (12.6%) rest under or among twigs
vi) There was no significant difference in the resting sites of males and females.

vii) There was no significant differences in the restings sites used by *typica*, *carbonaria* or *insularia* forms.

**PP32** And here are some of the moths.
While the results may be somewhat biased towards lower parts of the tree, due to sampling technique, I believe that they give the best field evidence that we have to date of where peppered moths spend the day.

**PP33** When you see moths like this, they are fairly easy to see. However, I have, through this work, come to understand why Clarke et al. (1985) wrote, *'In 25 years we have only found two* betularia *on the tree trunks or walls adjacent to our traps and none elsewhere".* So, **Can you see it?**

The results from resting site observations collected in the pilot year were used in deciding on release sites, with the proviso that the girth of the branch/trunk had to be less than 75cm to take the release arena.

**PP34** The frequencies of the *typica* and *carbonaria* forms for the seven years 2001-2007 were obtained by moth-trapping in Madingley Wood, 1.9km from the experimental site.
*Insularia*, which had a frequency of between about 6 and 10% throughout was excluded from analysis. Thus the frequencies of *carbonaria* here is the proportion of *typica* + *carbonaria* that were *carbonaria.*

**PP35** The basic results of the predation experiment are shown here, with the numbers of moths of each form available for predation and the numbers eaten given. The bottom line is that a significantly greater proportion of *carbonaria* were eaten than *typica*.

**PP36** A number of species of bird were observed preying on the moths: These included: robins, hedge sparrows, a lesser-spotted woodpecker, great tits, blue tits, blackbirds, starlings, wrens and magpies.

**PP37** From the data, the selection coefficient needed to account for the decline in *carbonaria* frequency for each year between 2001 and 2007 can be compared to the selection coefficient observed in the predation experiment for each year 2002-2007. Here, were bird predation to be a causative factor of changes in *carbonaria* frequency, compared to *typica,* the observed frequency in one year should be a consequence of the predation the previous year. So, I do not have predation data to account for changes from 2001-2, and the predation observed in 2007, should be predictive of frequencies in 2008.

However, we can look at two things here. First, the average selection against *carbonaria* over the period is not very different between that gained from the form frequency data and that observed in the predation experiment.

Second, the correlation between these for the five years for which we can make the comparison (that's 2002-2006) is rather high.

**PP38** I conclude that differential bird predation here is a major factor responsible for the decline in *carbonaria* frequency in Cambridge between 2001 and 2007.

So Tutt's hypothesis stands, and is once again supported by empirical evidence.

**PP39** There was another thing that I wanted to do when I started this work: that is, to be able to say with authority whether the peppered moth story should, or should not, be taught in biology class as an example of Darwinian evolution in action. For, as Douglas Adams (2002) pointed out in an essay in *The Salmon of Doubt*, "… Even today that persists as a slightly tricky problem if you are trying to persuade somebody who doesn't believe in this evolution stuff and wants you to show him an example – they are hard to find in terms of everyday observation.

But the peppered moth story is easy to understand, because it involves things that we are familiar with: vision and predation and birds and moths and pollution and camouflage and lunch and death. That is why the anti-evolution lobby attacks the peppered moth story. They are frightened that too many people will be able to understand.

I said I would have a few *mild* concluding remarks: Here they are.

**Hooper Book**

First, I would like to thank Judith Hooper for being the catalyst for me being here. I am sure that she never intended that her book would be such a boom to my research. She certainly didn't have a very high opinion of moth men. For example, she wrote: **PP40** "*Moth men have stunted social skills of the more monomaniacal computer hackers, going about with mis-buttoned shirts and uncombed hair, spouting taxonomic Latin*"

I'll let you judge.

More seriously, when I was at school in the late 1960s, I was taught about the physics, chemistry and geography of 'the greenhouse effect" as fact. According to the scientific laws of these subjects, global warming could not NOT happen. It has taken nearly 40 years for scientists to accumulate the empirical evidence of proof, so that even those with strong vested interests in denying global warming have had to concede that climate change is now a fact of life.

Similarly, Darwinian evolution is logical fact, and had to be even in 1859. Consider Darwin's four observations and three deductions, upon which selection theory is based.

**PP41**

Organisms produce far more offspring than give rise to mature individuals.
Yet, population sizes remain more or less constant.
Therefore, there must be a high rate of mortality.
The individuals in a species show variation.
Therefore, some variants will succeed better than others, and those with beneficial characteristics will be naturally selected to produce the next generation.
There is a hereditary resemblance between parents and offspring.
Therefore, beneficial traits will be passed to future generations.

Given these four observed facts and three simple, logical deductions, selection cannot NOT happen. And there are a tremendous number of examples of Darwinian selection in action, whether these be due to natural, sexual or artificial selection.

Yet, the majority of people in the world do not accept the fact of Darwinian evolution. Many people seem to need to believe that there is something more. Take Sir Arthur Conan Doyle's Sherlock Holmes **PP42**

**HOLMES BOOK**

Despite being an advocate of logic, Holmes thought it unthinkable that their wasn't some higher meaning to life. Now I believe that people's beliefs are a matter for themselves, as long as they don't try to tell me what to believe. There is a place for teachings of belief. But belief and faith are not scientific. They are not based on objectivity, hypothesis formation and testing, and experimental rigour. For example, fundamental creationism and intelligent design are not scientific theories. They are not testable. AND they have no place in science class.

I believe in the existence of god in the same way that Douglas Adams believed in the existence of god. Just as Terry Pratchett proved, through logical argument the existence of Father Christmas (well, actually it was the Hogfather), saying that if you can draw him, he must have existence, so Douglas Adams proved the existence of God. Moreover, he also proved that humans invented him or her.

It is not my place to tell people what to believe. But I know that we are making a horrendous mess of this planet, and I do not have faith in some supernatural intervention putting it right: No second coming; No helping hand from on high; No last minute redemption.

I caught my first butterfly when I was four, and started recording peppered moth forms when I was 10. I am getting old, and have spent my life in scientific enquiry and discovery. And it has been a **great** life!

*Until now*, for instead of the vision of a world made better by the appliance of science, I see a future of ever-increasing global problems. I probably won't see the worst of what's coming – but I fear for my children, who will face escalating problems of climate change, over-population, pollution, starvation, disease and conflict. And for their children and grandchildren, I have little optimism.

**PP** We need to address global problems now, and to do so with any chance of success, we have to base our decisions on scientific facts: and that includes the **fact** of Darwinian evolution. If the rise and fall of the peppered moth is one of the most visually impacting and easily understood examples of Darwinian evolution in action, it should be taught. It provides after all: **The Proof of Evolution.**