

**RUDIMENTARY TEMPERATURE EXPERIMENTS CONDUCTED
ON THE PUPAE OF ARGYNNIS AGLAJA (L.)
(LEP.: NYMPHALIDAE)**

J. C. WEIR

32 Paul Drive, Airth, Falkirk, Stirlingshire FK2 8LA

Abstract

Temperature experiments on *Argynnis aglaja* (L.) pupae are described. Specimens were exposed to warm (~25°C), cold (~5°C) and alternating warm and cold temperatures. Pupal mortality rates under each temperature treatment are given and the colouration of the resulting imagines is described and figured. It is tentatively concluded that the results are indicative of warm temperatures producing a general darkening of the imagines and cold temperatures the reverse. It is suggested that cold temperatures induce the production of either ab. *flavescens* Tutt or ab. *aurantica* Reuss. The variation displayed by this species in Britain is considered in light of this evidence and it is suggested that the results are concordant with the Hebridean race *scotica* Watkins being of adaptive origin and not due to direct environmental influences upon development.

Keywords: *Argynnis aglaja* (L.), temperature, colouration, development, *scotica* Watkins, *aurantica* Reuss, *flavescens* Tutt.

Introduction

Argynnis aglaja (L.) is a nymphalid butterfly of open grassland, heaths and glades, ranging throughout Europe and North Africa into Asia and the Orient (Higgins, 1975; Tolman, 1997). In the spring of 2014, I obtained larvae of this species originating from the Vale of Lindrick, Yorkshire, with the intention of carrying out temperature experiments on the pupae.

At the end of the nineteenth century, amid growing interest in the evolutionary, genetic and developmental basis of variation, a series of significant experiments revealed that exposure of the pupae of Lepidoptera to extremes of temperature could dramatically affect the imaginal colouration (Merrifield, 1890; 1891a; 1991b; 1892; Standfuss, 1900). Subsequently, the results of such experimental manipulations in many species have reproduced examples of naturally occurring aberrations, indicating the particular conditions leading to their production in the wild (Harmer, 2000; Yeates, 2015).

While the results of temperature experiments on Lepidoptera continue to be reported (e.g. Bailey, 2014; Bailey, 2015; Yeates, 2015), to my knowledge this species has hitherto not been subject to any such investigations. Therefore, while these experiments were conducted on a very small scale, and the inferences drawn from their results tentative, any relevant information is perhaps worthy of permanent record. The results themselves were so striking and unexpected that they warrant being brought before a wider audience.

Temperature experiments

Twenty larvae of *A. aglaja* were reared on *Viola tricolor* var. *hortensis* purchased from a local garden centre. Of these, 14 survived and pupated successfully. I divided the resulting pupae into three temperature treatment groups:

Treatment 1. Kept continuously at room temperature ($\sim 25^{\circ}\text{C}$). Four pupae.

Treatment 2. Kept at room temperature ($\sim 25^{\circ}\text{C}$) during the day and placed in the refrigerator at night ($\sim 5^{\circ}\text{C}$). Pupae were placed in the refrigerator at approximately 10pm each night and removed at approximately 10am each morning. Thus, they were alternately exposed to $\sim 25^{\circ}\text{C}$ and $\sim 5^{\circ}\text{C}$ for 12 hour periods. Five pupae.

Treatment 3. Kept in the refrigerator ($\sim 5^{\circ}\text{C}$) continuously for two and a half weeks. Thereafter, pupae were removed and kept at room temperature ($\sim 25^{\circ}\text{C}$). Five pupae.

All such treatments were initiated as soon after pupal formation as possible. After emergence, specimens were stored in a darkened cupboard for two days to ensure their wings had properly expanded and dried. They were then killed, using acetone vapour and set.

Results

The proportion of pupae from which imagines successfully eclosed in each treatment group was as follows:

Treatment	Pupae in sample	Number successfully eclosed
1	4	4
2	5	3
3	5	1

The different temperature treatments, within the limited size of the experiment, did appear to exert specific effects on the imaginal colouration of the individuals to which they were applied (Plate 1). In the following discussion the male and female illustrations given in Thomas and Lewington (2010) are taken as representing the typical colouration of this species in Britain, to provide a definite point of reference for any qualitative comparison with the experimental imagines. Wing vein and cell nomenclature follows the Rothschild-Jordan system (Miller, 1969).

Treatment 1

Dorsal: Specimens *a*, *c-d* have typical ground-colour, *b* has distinctly paler ground-colour, such that it approaches that between the terminal fascia and

the triangular subterminal marks. Typically the female possesses paler ground-colouration and *b* therefore appears to represent an accentuation of this character. Generally, black wing markings are either typical in extent (*a*, *c*) or slightly expanded (*b*, *d*). Specimen *d* displays asymmetrical colour pattern variation in the series of black costal bands on the (dorsally) left anterior wing, resulting in the bands appearing to blur and coalesce. Specimen *a* possesses blotches of bleached colour in smudges across some of the postmedian spots. These are symmetrical across the anterior wings but asymmetrical across the posterior, and are likely due to developmental scale defects. Specimen *b* showed some imperfect wing expansion, particularly in the posterior.

Ventral: Specimen *a* has typical colouration, though the (presumed) scale defect occurs also on the underside with convergent extent. In *b* the green markings on the posterior wings are extended. Both *c* and *d* possess typical colouration, and the dorsal asymmetric aberration on the anterior wing in *d* is not mirrored ventrally.

Treatment 2

Dorsal: Ground-colour paler than typical in both specimens *e* and *f*. As might be expected specimen *f* is paler than *e*, as typically female specimens have paler ground-colouration—this is indicative of the temperature treatment exerting a quantitatively uniform effect across the sexes. In specimen *e*, the central postmedian spot on the posterior wings is reduced in extent. Other than this point, the black markings of these specimens are typical.

Ventral: Both specimens *e* and *f* display typical ventral colouration.

Treatment 3

Dorsal: Ground-colour of specimen *g* much paler than typically, particularly on the posterior wings. Almost all black markings noticeably reduced in extent, particularly on the posterior wings. The basal patch of bronze scales is greater in extent than both typical individuals and all other bred individuals described here, extending into cell M_1-M_2 . On the posterior wings, the postmedian spots are very much reduced in size, some to near-vanishing. Curiously however, the subterminal triangular markings on these wings are extended in a proximal direction, in the manner of a typical female.

Ventral: The basal green markings on the posterior wings are paler than typically, and slightly reduced in extent. Silver markings are expanded to varying degrees though not excessively so. The silver streak along the dorsum of the posterior wing is broader and particularly prominent.

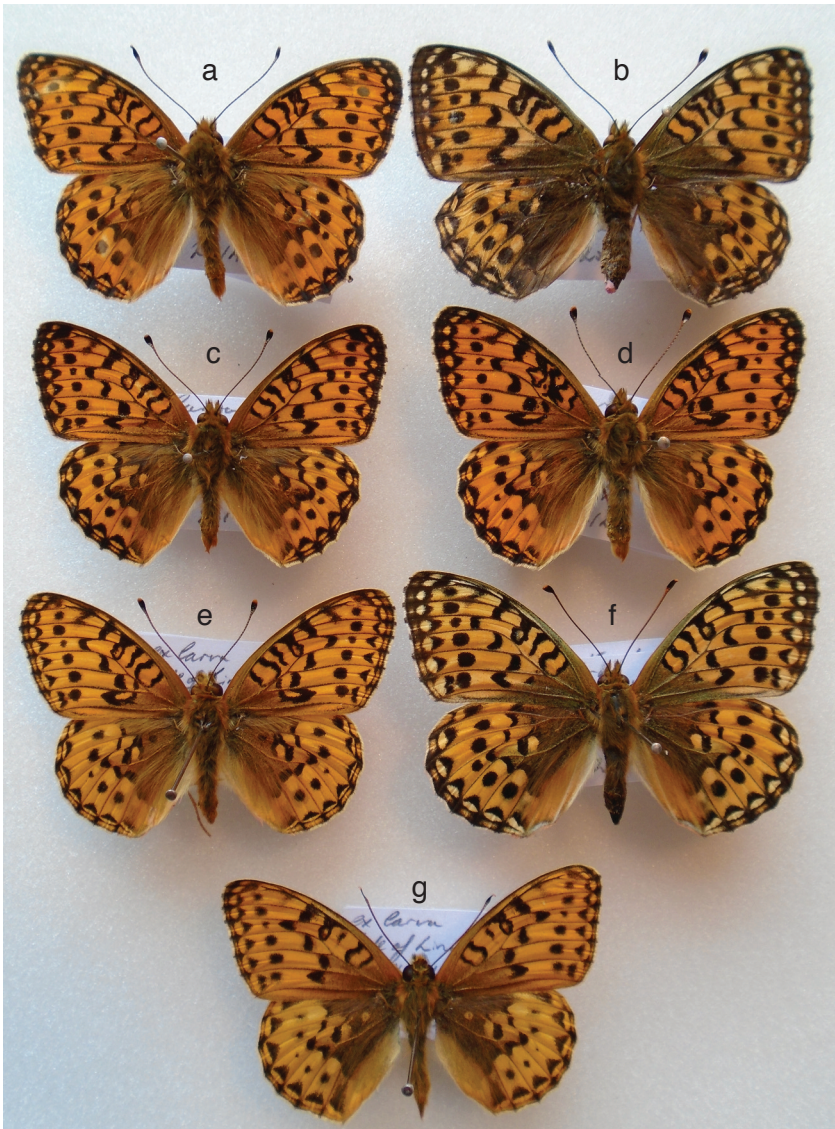


Plate 1. Results of temperature experiments on the pupae of *Argynnis aglaja*. Specimens *a-d* kept at constant $\sim 25^{\circ}\text{C}$ throughout pupal stage. Specimens *e-f* alternately exposed to $\sim 25^{\circ}\text{C}$ and $\sim 5^{\circ}\text{C}$ for 12 hour periods. Specimen *g* kept at $\sim 5^{\circ}\text{C}$ continuously for three weeks, before removal and storage at $\sim 25^{\circ}\text{C}$ until eclosion. Specimens *b, f* = ♀♀. Specimens *a, c-e, g* = ♂♂.

The effects of temperature on imaginal colouration

Bearing in mind the limited scope of these experiments, the results described above would seem to indicate that lower temperatures affect the development of the insect such that the ground-colour is much paler and the black wing markings are (generally) reduced in extent. Exposure to warmer temperatures had a slightly more mixed effect, where some individuals were somewhat darker in colour, and others were typical. Such a weaker response might be expected however, if the warm temperature to which the pupae were exposed ($\sim 25^{\circ}\text{C}$) was not so extreme as the cold temperature ($\sim 5^{\circ}\text{C}$); indeed, the far lower mortality among pupae reared at room temperature would seem to suggest that this is the case. Nonetheless, where specimens are aberrant (e.g. Specimens *b* and *d*), the aberration seems to lead to an expansion or disruption of the black wing markings.

Those individuals which most resembled typical specimens were those exposed to alternating warm and cool temperatures, as they might be in a natural situation from day to night. The slight disparity in colouration—that is, paler ground-colour—could be explained by the cool treatment temperature being more extreme than the corresponding “cool” temperature the species would naturally experience.

Species within the same family tend to display common imaginal colouration responses to exposure of the pupae to similar temperature extremes; for example, darkly coloured individuals of many species of the Pieridae may be induced by exposing the pupae to warm temperatures, and pale individuals by cool temperatures (Ford, 1953). Conversely, heat applied to pupae of nymphalid species results in paler specimens, while cold produces darker specimens (e.g. Ford, 1953; Yeats, 2015).

Thus, these results are particularly striking because they suggest that *A. aglaja* responds to exposure to these extremes of temperature differently from the majority of the Nymphalidae. Given this, the most parsimonious solution would have been to conclude that, due to the small experimental sample size, this represented a random error. And yet, this hypothesis is supported by the fact that not only do cold temperatures induce paler specimens but the corollary is also borne out—that exposure to warmth seems to tend in the direction of producing darker, more heavily marked specimens. Other instances of species displaying different colouration responses to that which generally prevails in their taxonomic family are not unknown; the colouration of imagines of *Leptidea sinapis* (L.), a member of the Pieridae, when exposed to extremes of temperature, is in the typical nymphalid pattern (Ford, 1953).

Variation in *Argynnis aglaja*

It may be convenient at this point to consider the variation in this species more generally, and how it might be viewed in light of the results presented here. Colour variants of *A. aglaja* tend to occur in Britain as either rare aberrants or geographical races and sub-species.

Aberrations

Howarth (1973) gives this species as possessing 95 named aberrations. Among these aberrants the overwhelming tendency is toward melanism, with considerable variation in the extent of the black dorsal markings; many names have been ascribed to the numerous variants of this species approaching total melanisation (Russwurm, 1978 and www.ukbutterflies.co.uk/aberrations.php?species=aglaja, accessed 10.v.2016). The apparently greater frequency with which melanic aberrations occur in *A. aglaja*, as opposed to pale or albino specimens, is potentially to be explained by its overwintering as a newly emerged larva (Thomas and Lewington, 2010). The larvae must wait until the onset of the warmer spring weather to begin feeding, such that pupation is postponed until late spring/early summer. One might contend that, at this period, any particular pupa is more likely to be exposed to unseasonal or “extreme” warmth, rather than cold—at least, cold temperatures at the level shown here to exert an effect (i.e. $\sim 5^{\circ}\text{C}$). Indeed, a pupa exposed to direct sunlight for a protracted period could develop an internal temperature significantly higher than that of the external environment. And, as suggested by the results above, treatment of pupae with higher temperatures appears to lead to extension of the black markings, and hence melanism.

Two named aberrations might potentially describe the single surviving specimen exposed to cold temperatures in this experiment (Plate 1 Figure g): ab. *flavescens* Tutt, where the ground-colour in the male is of an orange shade; or, ab. *aurantica* Reuss, where the ground-colour is a pale golden-yellow (Eeles, 2016). There is a degree of subjectivity in the interpretation of these descriptions and it may be that *aurantica* is a synonym of *flavescens*.

Geographical Variants

Within the British Isles, a geographical race of *A. aglaja* referred to as *scotica* Watkins occurs throughout the Inner and Outer Hebrides (Dennis, 1977). It is often treated as a sub-species, but in reality varies considerably in the degree and consistency of its derived characteristics and is thus better regarded as a race. The race is distinguished by possessing heavier black markings dorsally and ventrally, in both the male and female, darker green ventral colouration, expanded silver ventral markings and a greater wingspan (Watkins, 1923).

The results presented here are tentatively suggestive of this race being adaptive, and not the result of the particular environmental conditions which prevail across its distribution inducing developmental aberrations; for instance, given the *typical* colouration-response of exposing the pupae of nymphalid species to colder temperatures, one might expect that populations present in the north of the country would be composed of generally darker individuals. However, *A. aglaja* does not appear to share this common response.

Further evidence supportive of the adaptive nature of *scotica* includes its occurrence in Cornwall (Dennis, 1977), which has a generally higher average temperature than the Western Isles throughout May, June and July, during which the larvae pupate (www.metoffice.gov.uk/climate/uk/summaries/anomacts, accessed 20.v.2016). Also, Ford (1953) noted that a “very small pale” race of this species occurred on the island of Flodday, Outer Hebrides, which, were the *scotica* phenotype a result of the influence of the cooler ambient (Hebridean) temperatures upon pupal development, should not exist. Interestingly, subsequent investigation has suggested that this variant may now have been supplanted by *scotica* on the island (Barrington, 1999). In addition, *scotica* and typical *aglaja* meet on the Isle of Rhum, Inner Hebrides, and while some intermediates appear, both races seem to maintain their integrity within the island metapopulation (Ford, 1953)—this is highly suggestive of genetic evolution, not developmental aberration. However, Dennis (1977) has questioned precisely how sharply defined both races are on this island, stating that they are unlikely to be as discrete as Ford (1953) implied.

It is also interesting to note that *scotica* usually displays more prominent silver markings than typical *aglaja* individuals (Dennis, 1977)—a character shared by the single surviving cold treatment specimen from the experiments detailed above. It is therefore possible that this particular attribute of *scotica* is purely a result of environmental influence on development, or the adaptive exaggeration of a pre-existing developmental tendency; examination of *scotica* specimens from warmer localities would have to be made in order to resolve this point.

References

- Bailey, K. E. J., 2014. Exhibition report. *British Journal of Entomology and Natural History* **27**:101-104.
- Bailey, K. E. J., 2015. Exhibition report. *British Journal of Entomology and Natural History* **28**:77.
- Barrington, R., 1999. Notes on the dark green fritillary *Argynnis aglaja* L. (Lep.: Nymphalidae) from the island of Flodday, Outer Hebrides. *Entomologist's Record and Journal of Variation* **111**:57-61.
- Dennis, R. L. H., 1977. *The British Butterflies: Their origin and establishment*. Oxford, E. W. Classey.
- Ford, E. B., 1953. *Butterflies*. London, Collins.
- Harmer, A. S., 2000. *Variation in British Butterflies*. Hampshire, Paphia Publishing.
- Higgins, L. G., 1975. *The Classification of European Butterflies*. London, Collins.

- Howarth, T. G., 1973. *Colour Identification Guide to butterflies of the British Isles*. London, Frederick Warne.
- Merrifield, F., 1890. Systematic temperature experiments on some Lepidoptera in all their stages. *Transactions of the Entomological Society of London* **38**:131-159.
- Merrifield, F., 1891a. Conspicuous effects on the markings and colouring of Lepidoptera caused by exposure of the pupae to different temperature conditions. *Transactions of the Entomological Society of London* **39**:155-168.
- Merrifield, F., 1891b. Temperature experiments. *Psyche* **6**:196.
- Merrifield, F., 1892. The effects of artificial temperature on the colouring of several species of Lepidoptera, with an account of some experiments on the effects of light. *Transactions of the Entomological Society of London* **40**:33-44.
- Miller, L. D., 1969. Nomenclature of wing veins and cells. *Journal of Research on the Lepidoptera* **8**:37-48.
- Russwurm, A. D. A., 1978. *Aberrations of British Butterflies*. Oxford, E. W. Classey.
- Thomas, J. and Lewington, R., 2010. *The Butterflies of Britain and Ireland*. Dorset, British Wildlife Publishing.
- Tolman, T., 1997. *Field Guide to the Butterflies of Britain and Europe*. London, Harper Collins.
- Watkins, H. T. G., 1923. A new *Argynnis* race. *Entomologist* **56**:108-109.
- Yeates, M. A., 2015. Exhibition report. *British Journal of Entomology and Natural History* **28**: 78-79.
-
-

Emperor Moth *Saturnia pavonia* (L.) (Lep.: Saturniidae) assembling experiment

Three Emperor Moth cocoons rescued from systematic crow predation in autumn 2015 at Ordiquhill in Banffshire (*Ent. Rec.* **127**: 280-281) produced adults in May 2016. All were female, giving me the perfect opportunity to perform a little experiment that had long been on my mind.

This species is famous for the female's ability to attract males from a considerable distance by means of pheromones. Having observed this for myself on several previous occasions, I was struck by how accurately the male homes in upwind using the scent trail until he is within a metre or less of his target. After that, he often has considerable difficulty in actually locating the calling female, perhaps because her scent is now all around rather than directional. I have even seen males give up and fly away after failing to find her.

Whilst the female's scent is obviously the main attractant, could sight play a part in the last few centimetres? Does the male then need to locate the female visually? Might her eye-spots act like RAF roundels, enabling the male to identify her? To try and answer these questions, I made three decoy female Emperors out of thin white card, about 50% larger than life. On two of the cut-outs, the relevant markings were added using pencil and felt-tip pens, paying particular attention to the eye-spots, which were made especially prominent – a super-stimulus indeed. The third decoy was left unmarked, in case any pale object had the same effect.